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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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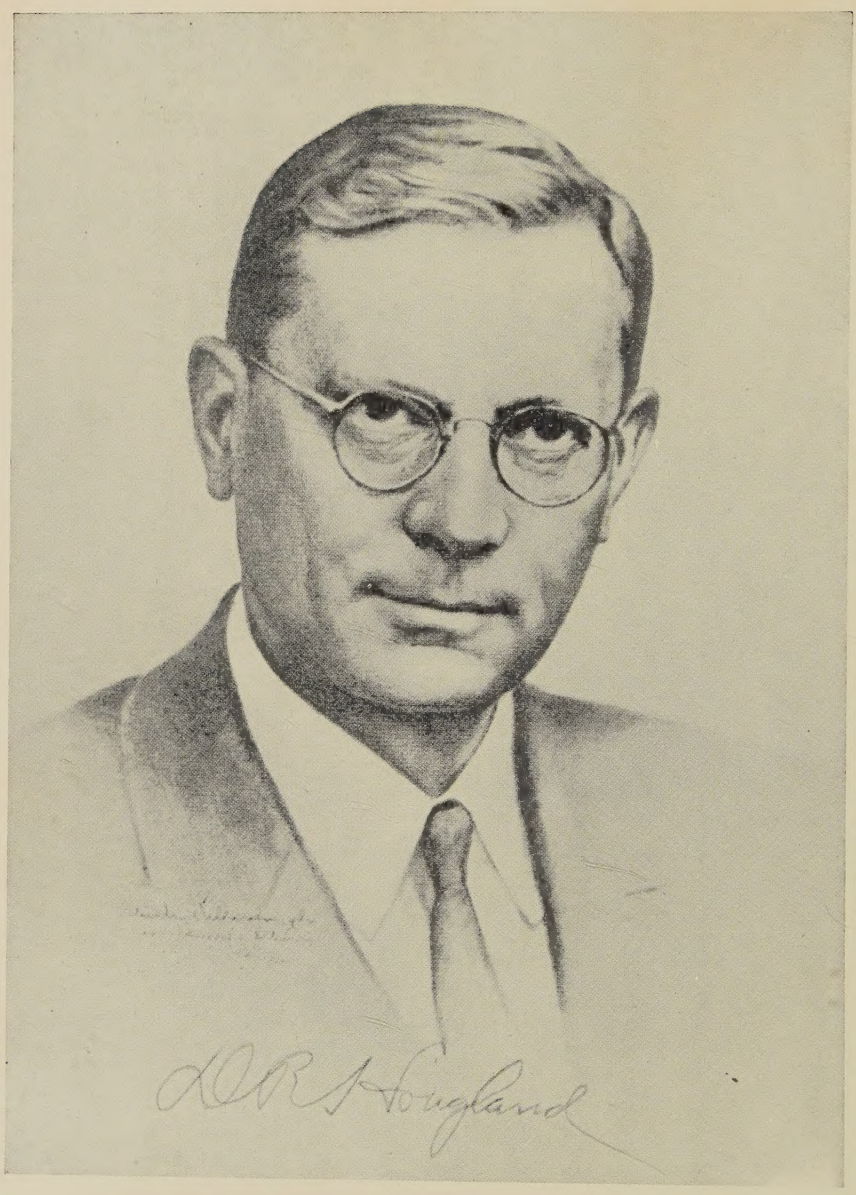


DENNIS ROBERT HOAGLAND
1884 — 1949

DENNIS ROBERT HOAGLAND 1884—1949

Dennis Robert Hoagland, Professor of Plant Nutrition in the University of California, one of the most distinguished plant physiologists and the world's leading authority in the broad field of plant and soil interrelations, died in Oakland, California, on September 5, 1949.

For over thirty years, Professor Hoagland's life and work exerted a profound influence on the evolution of plant and soil science in America and abroad. But as with most great men, the totality of his achievement is greater than the sum of his impressive individual contributions. He personified the qualities of mind and character which led some to observe that the scientist with a broad outlook on his work and the world around him is among the finest products of Western civilization.

D. R. Hoagland spent the first eight years of his life in the town of his birth, Golden, Colorado. In his own words "this was a small town, with all the narrowness of small town life. Something of the frontier spirit remained, however. The history of Golden was linked especially with the development of mining in Colorado. The foothills and mountains were near and familiar. My home was one of moderate comfort according to the standards of the time and place". All of his later childhood was passed in the city of Denver, where, except for the first two years of grammar school, he received his primary and secondary education before entering college. He graduated from East Denver High School in 1903.

Even in his early years he possessed an extreme fondness for reading, a trait which was to remain with him for life. Again, to quote him — "At the age of nine or ten a most cherished Christmas gift was a dictionary. My reading was indiscriminate: novels,

boy's stories, history. I was particularly attracted by stories which attempted to present scientific knowledge or speculation, such as 'Twenty thousand leagues under the sea'." In school he showed great promise by the uniformly excellent work in classes and a genuine interest in many studies extending beyond assigned work. Serious illness in childhood adversely affected his eyesight, and this handicapped his participation in sports.

It was during his high school days that he first acquired a definite interest in science "as a result of discussion of the theory of evolution which was condemned at that time by many ministers of the City; .... my thinking was greatly stimulated by the ideas and points of view of biology with regard to man's place in nature".

H o a g l a n d left Denver to enter the then new but already renowned Stanford University, where he elected chemistry as his field of study. He graduated from Stanford in 1907 and was elected that year to Phi Beta Kappa and to the Chemistry Honor Society, now Phi Lambda Upsilon. An amusing anecdote of the Stanford period which he was fond of telling in later years was a "slightly exaggerated" report of his death in the great San Francisco earthquake in 1906, published with a suitable obituary by a Denver paper.

After graduation he first elected to go on with graduate work in chemistry at Stanford and was for one semester a graduate assistant in physical chemistry. In January of 1908, however, he took a fateful turn in his career by accepting a position as an instructor and assistant in the Laboratory of Animal Nutrition at the University of California. The new position brought him for the first time in professional contact with biology and took him to Berkeley, where, except for a few short gaps he was to spend the rest of his life. Perhaps it was highly significant for his future life long activity in the field of plant nutrition that his introduction to biology was through the field of animal nutrition. He entered that field at a time when his training and skill as a chemist were welcome and useful but by no means decisive in graining a thorough understanding of the essentials of nutrition. There were of course, during that period, large areas of empiricism and sheer ignorance regarding the effects of different foods on nutrition and health and this general atmosphere might have very well helped him in acquiring the outlook of a biologist which he so thoroughly displayed in his

later work. He was spared the experience which some chemists undergo when first turning to biological pursuits — the failure to take into account the complexity of biological systems and the resultant predilection to oversimplification.

Hoagland remained in the field of animal nutrition and biochemistry from 1908 to 1913. In 1910 he joined the Consulting Referee Board of Scientific Experts of the United States Department of Agriculture. This board was established to study the effect on health of various chemicals used in food processing and as preservatives, a question of national concern raised by the administration of the then recent Pure Food and Drug Act. Hoagland acted as expert chemist and was associated with Dr. Alonzo Taylor in his work on the toxicity of aluminum, copper, and sulfur compounds. The experimental subjects were the so-called poison squad, a group of volunteer premedical students at the University of California and the University of Pennsylvania. His assignment took him to Philadelphia and made it possible for him to attend lectures as a graduate student in the University of Pennsylvania.

After the completion of the work of the Referee Board in 1912, Hoagland accepted a graduate scholarship to work in animal biochemistry with Prof. E. V. McCollum in the Department of Agricultural Chemistry at the University of Wisconsin. This was another crucial event in his life. In later years Hoagland looked back on the year of graduate study under Prof. McCollum as his real inspiration for a life devoted to scientific research. In 1913 he received his Master's Degree with a thesis on "The influence of sodium benzoate on the synthesis of urea". In the same year he published with E. V. McCollum a series of three papers on the "Endogenous Metabolism of the Pig as modified by various factors". These publications profoundly altered the current ideas on an important phase of animal metabolism and are still quoted as an authoritative statement on the process involved.

In 1913 Hoagland had thus completed a period of five years of continuous work in animal nutrition and had become associated with an inspiring teacher and leader in the field. There would normally have been every expectation that he would continue in this field which was then on the eve of revolutionary advances. Circumstances intervened, however, and Hoagland

abandoned the subject in which he had already amassed appreciable experience, to enter upon a new field of activity. For this, plant science will always remain indebted to Professor John S. Burd, then Head of the Division of Agricultural Chemistry in the College of Agriculture of the University of California. In 1913, Professor Burd invited Hoagland to come to Berkeley as an Assistant Professor of Agricultural Chemistry. Acceptance followed and with it the initiation of a scientific activity in a field in which he labored with devotion and distinction till the end of his days.

The appointment in the Division of Agricultural Chemistry of the University of California was a real opportunity to one who had already acquired an attachment for fundamental research. The department, under the leadership of Professor Burd, was chiefly interested in problems of fertilizers and soil chemistry. Practical and immediate as these questions appeared, Professor Burd had the vision to encourage the search for principles rather than *ad hoc* solutions which were thought by some to be attainable by a mere multiplicity of fertilizer field plots. A fortunate circumstance was also the early connection of Hoagland with teaching. It seems legitimate to speculate that the then large areas of ignorance in the field of plant and soil interrelations were sharply illuminated by penetrating questions from students.

The interruption of commerce in World War I focused attention on American dependence on German sources of potash fertilizers. There was considerable interest in exploring domestic sources of potassium and the giant kelps of the Pacific Coast were suggested as a possible source of supply. Hoagland undertook a systematic study of the inorganic and organic composition of kelps and reported his findings in three papers. His conclusions about the possibility of commercial exploitation of these organisms were not very encouraging but this research led him into a field of investigation in which he acquired a life-long interest and international renown: the accumulation of ions by plants. In 1915 he wrote in a paper dealing with the composition of kelps: "Of far greater interest to the plant chemist and physiologist is the study of the chemical composition and metabolism of these remarkable plants (rather than their economic importance). Their selective action on certain elements contained in sea water is very striking". He was particu-

larly impressed with the remarkable ability of these plants to accumulate potassium and iodine many times in excess of the concentrations found in sea water.

The second of his early research activities concerned itself with the effect of hydrogen ion concentration on plant growth. This was the era of "hydrogen ion concentration" in biology, when great expectations were centered around the introduction of the hydrogen electrode for the measurement of pH of biological systems. One of the accepted tenets of agricultural teaching in those days was that plants could only grow well at neutral or slightly alkaline reactions. It was with great surprise therefore that he discovered that barley plants made excellent growth in nutrient solutions at pH 5. It seemed that the chief importance of hydrogen ion was in relation to other ions rather than in its direct effect on the plant. It was also apparent that the inherent complexity of soils rendered untrustworthy many sweeping generalizations based on local conditions. To his orderly and critical mind it became clear that the complex problems of soil and plant interrelations must be studied by techniques which permitted rigid experimental control and the isolation of individual variables. This he expressed as follows: "Scientific research on soils and plants must place great reliance on the results of experiments conducted in the laboratory or greenhouse. The objection often raised to such investigations is that they are artificial and do not imitate field conditions. This is very properly the case, otherwise the requisite control would be absent. There can be nothing mysterious about field conditions. They are simply more variable and uncontrolled than soil cultures in pots or tanks".

He gave expression to his convictions about the value of artificial culture methods in plant research by developing to a very high degree the water-culture technique for growing plants, which has since, for over three decades served as a powerful and discriminating tool for studying fundamental problems in plant physiology at Berkeley. Under the influence of the soil solution studies conducted by Professor Burd, Hoagland devised a culture solution to imitate a soil solution obtained from a soil of proven fertility. This "Hoagland's solution" became, and remains to this day, a household term in laboratories of plant physiology throughout the world. The formulation of his culture solution was accompanied by a study of certain basic concepts such as concen-

tration versus total supply of ions, specific proportions of nutrients and the like which underlie the use of nutrient solutions. He critically examined and rejected the idea of an "optimum nutrient solution", a point of view which was fully corroborated by later work.

It was inevitable that his early researches into plant and soil chemistry would lead him to a conviction that the study of soil and plant interrelations could be pursued most auspiciously by a closer union between workers in soil chemistry and plant physiology. In his own words: "Soil chemistry and plant physiology cannot be divorced and the practical art of soil management must have as its scientific basis a definite concept of the chemical and physiological interrelation between soil and plant". This point of view already had strong advocates in the University of California College of Agriculture in the persons of the late C. B. Lipman, then Head of the Division of Soil Chemistry and Bacteriology, and J. S. Burd, Head of the Division of Agricultural Chemistry. In 1922 an administrative rearrangement in the College of Agriculture resulted in the merger of the two groups into a new Division of Plant Nutrition, and Hoagland was designated the Head of the new Division.

After the organization of the Division of Plant Nutrition, Hoagland returned with renewed vigor to the problem which attracted his attention in kelps — the absorption and accumulation of ions by plants. The quality of the immense contribution which he, with his collaborators and associates made to this fundamental process of plant physiology can only be judged against a background of the ideas and concepts which prevailed when his work got under way. There was no clear view as to how inorganic elements are absorbed by plants. Discussions of this subject were usually invoking such concepts as osmosis, permeability, antagonism, none of which as he later clearly demonstrated could explain the absorption, accumulation, and retention against a concentration gradient of anions and cations by plant cells. The prevailing confusion and uncertainty of thought at that period is illustrated by such contemporary theories as that the absorbing power of plants is contingent upon the reaction of cell sap and governed by the law of mass action. To explain potassium accumulation in plant cells it was sometimes assumed that this ion was precipitated in the vacuole.

Hoagland opened the study of ion absorption by using intact

barley plants. Promising as the early results were, he readily perceived the advantages of working with a unicellular organism from which relatively pure vacuolar sap could be obtained. At the suggestion of the late Dr. Nathaniel Gardner, he selected the fresh water alga, *Nitella*. This work clearly demonstrated that absorption of ions by plants is a metabolic process and not a question of simple permeability. For example, absorption and accumulation of bromide could take place in *Nitella* only under the right metabolic conditions — proper temperature and illumination. The accumulated ions were not precipitated in the sap but remained as free ions, the concentration of electrolytes in the *Nitella* sap being twenty five times greater than that in the surrounding medium. Although the concept of „ion antagonism" had no strict applicability to the normal process of nutrient absorption, there was clear evidence of reciprocal effects of one ion on the absorption of another, as, for example, potassium on calcium, and nitrate on phosphate. Here was painted in bold strokes an outline of a theory which at once correlated a host of observations in the field and laboratory, and made it possible to approach an infinitely complex subject with principles instead of mere empirical observations.

The next important phase of his researches on ion accumulation brought him closer to the system which was uppermost in his mind — the absorption of nutrients by the roots of higher plants. The broad outlines of a theory were already available from his previous work. What was needed was the formulation of an adequate experimental approach to the different system. In the solution of this problem, Hoagland displayed in a most striking manner his remarkable gift for resolving a complex question into its essential components and arranging them unerringly in an order of relative importance. He was always mindful of the inherent complexity of biological systems, their adaptive properties to varying conditions, and hence was distrustful of the spectacular short-cut or facile generalization unless it was supported by solid experimental evidence. As far as salt absorption by roots was concerned, he decided in the late 1920's that the most important prerequisite for future work was the development of a technique which would make available biological material of a definable and reproducible physiological status. Only in this manner could the various conflicting observations be resolved and a systematic effort directed toward

the elucidation of factors underlying salt absorption by roots.

He concluded that the fundamental questions of ion absorption by root cells can be studied most effectively by eliminating, during a brief experimental period, the complications of shoot and root relationships. He concentrated therefore on excised roots, obtained from plants grown under experimental conditions designed to yield, subsequent to the removal of shoots, root systems with a high capacity for salt absorption. Among the essential conditions for a high capacity for ion absorption by roots were a low salt and a high carbohydrate status. Under these conditions the excised roots were found to be "the most active salt-accumulating system so far (1936) investigated".

Once the tedious but indispensable job of evolving a suitable physiological technique was completed, progress was rapid gratifying. An impressive array of data was amassed over the years on the influence of temperature, light, hydrogen ion concentration, ion selectivity, concentration of anions and cations, differences in rates of absorption, and the effect of one ion on the absorption of another ion of the same or opposite charge. A striking correlation was obtained between the supply of oxygen and the absorption of salts by roots against a concentration gradient. A solid scientific foundation was thus laid for the understanding of the importance of aeration in soils and for the interpretation and prediction of a multiplicity of plant responses to fertilization treatments, and other chemical changes in the soil.

The final phase of his investigations on absorption of ions occurred just before and during World War II. With excised barley roots he studied the effect of reversing the external concentration gradient on ion entry and the influence of inhibitors on ion accumulation. He also returned to *Nitella*, this time employing radioactive isotopes — a sensitive technique which permitted the separation of the two stages of ion absorption by cells; from the external medium into the cytoplasm, and from the cytoplasm into the vacuole. The fundamental conclusion, which he and his colleagues reached almost two decades earlier, that ion accumulation is a metabolically linked process, was confirmed. With the use of radioisotopes it was possible to show that ions first accumulate in the cytoplasm but subsequently they migrate to the vacuole by a process akin to secretion, until the vacuolar concentration becomes higher than

that in the cytoplasm and decidedly higher than that of the external dilute solution.

Dominant as his life-long interest in the process of ion absorption was, it by no means prevented him from pursuing with vigor other phases of plant nutrition to which he, with his associates, made outstanding contributions. He studied by means of different techniques the upward movement and distribution of inorganic solutes in the plant. In the late 1930's, when radioactive isotopes from the cyclotron became available, he recognized at once their serviceability and used them in resolving some of the hitherto perplexing uncertainties as to the path of ion movement in higher plants. In a series of experiments on the relation between the movement of solutes and absorption of water by plants, he again demonstrated with masterful clarity the extent to which the two are related as well as independent of each other.

H o a g l a n d was keenly aware of the immense importance for physiological studies of the control of light, temperature and humidity as well as of the culture medium. He was instrumental in securing an installation for the control of environmental factors in Berkeley, it being one of the first in America. He envisaged "the development of a quantitative plant physiology in relation to mineral nutrition" when it will be possible "to do exactly the same thing twice".

A tribute to the catholicity of his interests and the breadth of his understanding is that preoccupation with precise laboratory experiments and insistence, when needed, on an exact though "artificial" culture condition, in no way diminished his appreciation of the importance of direct studies on soils and on crops grown in soil. In his own words: "Viewing the field as a whole, the attack on problems of plant nutrition assumes wide dissimilarity of method. At one time, the appropriate tool may be a spade or a soil auger; at another time a highly specialized and refined tool of physics or chemistry may be required. For some purposes, crude measurements in terms of pounds or ounces may suffice; for other purposes, a thousandth of a milligram or even much less than that is of consequence". This heterogeneity of experimental method, was necessary, he felt, because of the extraordinary complexity of the plant-soil-atmosphere system which the investigator of plant nutrition explores. To quote him again: "Inherent in the green plant itself

are all the complexities common to living organisms and to these must be added the complexities of the soil medium in which the plant is anchored and finds some of the substances essential for its nourishment .... Finally, the whole system of plant and soil is subject to the influence of another constellation of factors in the atmospheric environment — light, temperature, humidity, rainfall, air movement, carbon dioxide and other gaseous components of the atmosphere".

His own work on problems of soil chemistry insofar as it affects the nutrition of crops grown in the field, was especially concerned with zinc, potassium, and phosphate deficiencies of fruit trees in California. In this he entered, in the late 1920's, into a harmonious cooperation with Professor W. H. Chandler of the Pomology Division, and the resultant diversified investigations which embraced field, greenhouse, and laboratory experimentation will stand out as models of cooperation among different divisions in an agricultural experiment station. In a concerted attack on a complex problem, each division contributed what it was best equipped and qualified to do. The result of this collaboration was the clear identification of "little leaf" of fruit trees as a zinc deficiency and its reproduction under controlled greenhouse conditions. The importance of these studies to California agriculture is shown in the fact that at the present time, zinc is second only to nitrogen as a fertilizer element for orchard crops. The investigation of potassium nutrition of fruit trees had a bearing on "prune dieback", another nutritional disease of fruit trees in California. An intensive chemical investigation followed into the question of potassium absorption in relation to replaceable, non-replaceable, and soil solution potassium. His interest in the nutrition of fruit trees continued, and in 1940 he produced, under controlled conditions, the first molybdenum deficiency symptoms in fruit trees. It was also under his influence and encouragement that intensive studies into other aspects of micronutrients have been undertaken in the Division of Plant Nutrition.

In the middle 1930's, immense public interest was aroused in the commercial possibilities of nutrient solution techniques for crop production. To one who, like Hoagland, had been using nutrient culture techniques for over a quarter of a century for experimental purposes, this newly aroused enthusiasm appeared

to be wholly without scientific foundation. He preferred to remain aloof from this development and to await its expected demise, but to save embarrassment to his institution, he undertook, in response to a direct request, a careful study of the inherent productive capacity of a fertile soil as compared with artificial nutrient media. The results, which were the only ones of their kind available, not only provided a scientific basis for evaluating diverse factors, but also served as a foundation for the preparation of a popular circular which was distributed in response to tens of thousands of requests which came to the University of California. It is safe to say that this publication exerted great influence in wide circles in restoring a proper perspective with regard to crop production in soil.

In summarizing Hoagland's contribution to plant nutrition, it is pertinent to record that he did not regard the field as a unified discipline. In his own words: "An essential condition in the field of study of Plant Nutrition .... is the uniting together, although not according to any rigid pattern, of persons of varied technical interests, in a research program that has a common objective". While he himself was mainly engaged in what is known as mineral nutrition of plants, he was keenly aware of the importance of the biochemical aspects of plant nutrition. He wrote "The inorganic elements derived from the soil constitute only a small percentage of the dry weight of a plant and one of the most important objectives of research in plant nutrition should be an understanding of the mechanisms by which these inorganic components become directly incorporated into the organic compounds synthesized by the plant, or activate the enzymes which catalyze the syntheses and breakdown of organic compounds". Under his leadership the Division of Plant Nutrition became an outstanding center of research, encompassing a diversity of investigations seldom encountered within the confines of a rather small administrative unit. He was always mindful, however, of "the broad common objective" and exerted great personal influence in guiding the thoughts and interests of his associates into channels which would ultimately lead to the common goal.

Hoagland's entire life as a productive scientist was spent in association with agricultural research. He had a profound faith in the importance to agriculture of fundamental research, without thought and expectation of immediate practical returns. He was

fully aware of the often wide gap between the laboratory result obtained under controlled conditions and the field application complicated as it is by a multiplicity of factors. He sought principles in the laboratory to help in solving the practical problems of the farmer in the field, but was never prone to seek in laboratory observations a facile generalization which would solve field problems with dazzling simplicity. He was conscious not only of the promise but also of the limitations of science in agriculture, and his thoughts on this subject ranged beyond mere material achievements. His philosophy is best described by two quotations which he himself selected in a public address as best representing his own views on these matters. The first is from Sir Daniel Hall: "Great are the achievements and still greater the possibilities of agricultural research, but we must recognize that there are limitations to the effect of science upon agriculture which do not hold for the other industries. In the first place, in agriculture we are dealing with a living organism and the amount of control that we have obtained over plant or animal, over that stubborn essence we call life, is far less than we can exercise over inanimate nature, over iron or cement, over even the ether or the atom. When we attack vital problems we find that we cannot speed up processes or enlarge the unit in the way we can deal with the dynamo or spinning frame. It still takes the wheat plant six or nine months to develop, and cows bring forth their calves neither more quickly nor more numerously for us than they did for Abraham. We see no way of growing three or four crops a year under temperate climatic conditions. The organisms we are dealing with will go through their cycle and you cannot hurry them. When you start hustling you find you let in secondary troubles of all sorts".

The second quotation is from Sir John Russell, the now retired Director of the Rothamsted Experiment Station: "And that leads to my last point. What is the purpose of it all? Team work, cooperation, the great expenditure of time and money now being incurred in agricultural science and experiment — these are justified only if the end is worthy of the effort. The nineteenth century took the view that agricultural science was justified only insofar as it was useful. This view we now believe to be too narrow. The practical purpose is of course essential; the station must help the farmer in his daily difficulties — which again necessitates cooperation, this

time between the practical grower and the scientific worker. But history has shown that institutions and investigators that tie themselves down to purely practical problems do not get very far; all experience proves that the safest way of making advances, even for purely practical purposes, is to leave the investigator unfettered. Our declared aim at Rothamsted is to discover the principles underlying the great facts of agriculture and to put the knowledge thus gained into a form in which it can be used by teachers, experts, and farmers for the upraising of country life and the improvements of the standard of living.

"This wider purpose gives the investigator full latitude, and it justifies an investigation whether the results will be immediately useful or not — so long as they are trustworthy. For the upraising of country life necessitates a higher standard of education for the countryman; and education based on the wonderful book of Nature which lies open for all to read if they but could. How many farmers know anything about the remarkable structure of the soil they till, of its fascinating history, of the teeming population of living organisms that dwell in its dark recesses; of the wonderful wheel of life in which the plant takes up simple substances and in some mysterious way fashions them into foods for men and animals and packs them with energy drawn out of the sunlight — energy which enables us to move and work, to drive engines, motor-cars, and all the other complex agencies of modern civilization? No one knows much of these things; but if we knew more, and could tell it as it deserves to be told, we should have a story that would make the wildest romance of human imagination seem dull by comparison and would dispel forever the illusion that the country is a dull place to live in. Agricultural science must be judged not only by its material achievements, but also by its success in revealing to the countryman something of the wonder and the mystery of the great open spaces in which he dwells".

As an administrator of a department, H o a g l a n d was anxious not to fritter away his time and energy on the minutiae of scientific housekeeping and administrative procedure but sought to discharge his responsibility to the University by concentrating on the encouragement and development of the broad aspects of his field of science. In relations with his colleagues, young and old, he always comported himself with the courtesy and understanding of the

true scholar for the scientific interests and activities of others. He was singularly free from any notion that notable advances in fundamental knowledge can be attained by administrative direction.

He was an ideal collaborator in scientific research, never seeking dominance for his own point of view but always inviting a free flow of ideas. He was meticulously careful in giving credit where credit was due and was generous in acknowledging the contributions of his associates. He was a master of clear exposition and had a real feeling for style. Although writing came to him easily, he always preferred to defer publication of results until the evidence was carefully assembled and checked.

H o a g l a n d's chief characteristic whether in scientific matters or personal relationships was integrity and objectivity of outlook. In evaluating scientific evidence he had the „four things" which S o c r a t e s said "belong to a judge: to hear courteously, to answer wisely, to consider soberly, and to decide impartially". He applied the same critical standards to his own work as to that of others. He accepted no blanket authority in science and expected every single piece of research to stand on its own merits regardless of its source. In personal relationships he possessed the engaging quality of always seeking the good in men and their work. In approaching students he had a rare gift of making them sense the good will and confidence in their ability, with which he always credited them at the start. His students were spurred to special efforts by a desire to prove themselves worthy of his trust. H o a g l a n d's confidence and friendship once given was not easily withdrawn. Never demonstrative in manner, he had a deep loyalty and an unfailing concern for the welfare of his friends and associates.

H o a g l a n d was the not altogether common scientist who carried the scientific mode of thinking outside his own specialty into diverse areas of human thought and achievement. He maintained an active interest not only in many departments of science but also in fundamental questions of education and in the social and political problems of the contemporary scene. It was a stimulating experience to witness his well-stocked mind analyze a complex issue with clarity and insight which unerringly penetrated to the root of the matter. Whatever the subject may be, his discussions had a special flavor of emotional and intellectual maturity which

excluded bias and preconceived notion. In the seventeen years during which the writer was associated with him as a student, colleague and friend, he never once heard him discuss a person or an issue, scientific or otherwise, except in a fair and objective manner.

It was only natural that his accomplishments, his qualities of mind and character, gained him wide recognition and many honors. His counsel was sought and valued within his own University, to which he gave unstintingly of time and effort in many arduous administrative assignments, usually at the expense of his own leisure and in later years at the expense of his health. In 1934 he was elected a Member of the National Academy of Sciences. The American Society of Plant Physiologists, which was his closest scientific affiliation, bestowed upon him its highest honor by granting him, in 1929, the first Stephen Hales Award in recognition of his outstanding research contributions. He was also elected President of the Society and occupied other important offices in it.

The high esteem in which he was held by plant physiologists throughout the world is attested by his recent appointment as President of the Section of Plant Physiology at the forthcoming VIIth International Botanical Congress to be held in Sweden in 1950. Unfortunately, poor health forced him to decline this international honor.

Many other societies in which he held membership honored him by election to presidency: Western Society of Soil Science (1924); Western Society of Naturalists (1931); Botanical Society of America, Pacific Division (1929); American Association for the Advancement of Science, Pacific Division (1941). He served as Consulting Editor for Soil Science, American Journal of Botany, Plant Physiology and Plant and Soil. He took a prominent part in the organisation of the Annual Review of Biochemistry, serving from its very inception on the Advisory Board and later on, on the Board of Directors of Annual Reviews. He was a member of the Board of Collaborators for the U.S. Department of Agriculture Salinity Laboratory at Riverside, California, and the Soil, Plant and Animal Nutrition Laboratory at Cornell University.

In 1940 the American Association for the Advancement of Science awarded him the $ 1000 prise for an outstanding paper presented at the Philadelphia meetings. In 1942 he was invited by Harvard University to give the John M. Prather lectures. The lectures were

later published in book form, and constitute the only book which he found leisure to prepare.

His own colleagues in the various Faculties bestowed upon him the highest honor within the University, by appointing him in 1942, Faculty Research Lecturer.

Hoagland was never in robust health, but a strong sense of duty compelled him to carry on without a thought for his own personal well-being. The last four years of his life were marred by serious illness. He carried on with courage and determination to within the last few months of his life when his eyesight failed him almost completely. This latter blow was the severest of all, for throughout his life he enjoyed reading on a wide variety of subjects. It was characteristic of the man that once he gave up hope of regaining his health, he requested retirement from the University, ignoring the serious financial adjustment that such a voluntary step would involve. He was officially retired on July 1, 1949, and died almost two months later.

In 1920, he married J e s s i e A. S m i l e y. She died suddenly of pneumonia in 1933. He was left with the responsibility of bringing up three young boys. His three sons, his Mother and a brother survive him.

The scientific influence of Professor H o a g l a n d's life has not come to an end with his death, but will continue through the deep impression which he made on the minds and hearts of his students and friends.

Berkeley, California
September 1949.

DANIEL I. ARNON.

MAGNESIUM DEFICIENCY IN FRUIT TREES ON SANDY SOILS AND CLAY SOILS IN HOLLAND

by D. MULDER

Laboratory of „Zeelands Proeftuin" Wilhelminadorp near Goes, The Netherlands

Introduction. Until recently, magnesium deficiencies in Holland were only known in agricultural and vegetable crops grown on sandy soils.

According to unpublished results of Miss Ir. J. ter Kuyle however, magnesium deficiencies have also been proved to occur in fruit trees on sandy soils. Details of this work are given below. In 1946 symptoms of magnesium deficiency were also to be seen on fruit trees growing on clay soils. The symptoms were very variable and this variability caused confusion with symptoms of potash deficiency and spray-damage. The causal factor of this deficiency on clay soils appears to be the prolonged use of potash fertilizers without the application of sufficient magnesium. In many cases the percentage potash contents had increased to a value far above the percentage of clay particles in the soil. It is considered to be most favorable for the good health of the plants when the potash figure (the number of thousandth parts of a percent) does not exceed the percentage of clay particles.

Literature. In England, the United States and Australia numerous research workers have studied the relationship between potash and magnesium nutrition. It has been shown that in the case of a potash deficiency, the magnesium content increases, and in the case of a magnesium deficiency the potash content increases. If the Mg content of a leaf falls below 0,40% MgO (dry matter) then Wallace (1940) expects magnesium deficiency symptoms. As soon as the potash content falls below 1,00% K_2O, potash deficiency symptoms can be expected.

From the review of the literature by Goodall and Gregory (1947) we can obtain some idea as to the contents of normal and diseased leaves of apple trees.

TABLE I

Magnesium contents of apple leaves (% D.Wt.Mg) according to the literature reviewed by Goodall and Gregory (simplified) (1947)

Location of leaves on shoot	Healthy			Diseased		
	minimum & maximum	average	average of averages	minimum & maximum	average	average of averages
top	0,32–0,78	0,55		0,17–0,38	0,27	
middle	0,2 –0,5			0,02–0,3		
,,	0,1 –0,2	0,22	0,33	0,07–0,19	0,14	0,17
,,	0,2 –0,3			0,09–0,23		
,,	0,1 –0,27			0,08–0,2		
base	0,2 –0,4			0,05–0,17		
	0,1 –0,3	0,22		0,05–0,19	0,12	
	0,15–0,19			0,13–0,18		

Leaves of healthy trees contain on the average, twice as much magnesium as leaves of diseased trees. Both in diseased and healthy trees the content of the top leaves is twice as high as that of the basal leaves. According to these figures, the magnesium content of healthy basal leaves is almost the same as that given by Wallace as the lowest concentration for healthy leaves, namely 0,22% Mg. Boynton and Burrell have found approximately the same. Little is known about the potash contents. Only the contents in case of a deficiency and the normal content are given. No figures have been published concerning the percentages in cases of magnesium deficiency.

It is very probable that contents above 1,3% K (approximately double the quantity at the deficiency level) have to be considered as abnormally high.

The figures of Boynton and Burrell (1944) and those of Kidson Askew and Chittenden (1940) can be compiled as follows: See Table II next page.

The symptoms. Potash and magnesium deficiencies often result in contrasting symptoms on the tree.

A potash deficiency causes necroses along the margin of the leaf, whereas a magnesium deficiency is characterized by necrosis of

Magnesium deficiency in fruit trees

1. Cherry
2. Pear: Louise Bonne d'Avranches
3. Apple: Glorie van Holland
4. Apple: Manks Codlin
5. „ Reinette van Zuccamaglio
6. „ Freiherr von Berlepps

TABLE II

Magnesium- and potash contents of apple leaves (as extracted from the literature)

	K %	Mg %	Ca %
Boynton			
Excess of potash	1,36–2,40	0,08–0,13	1,34
Potash deficient	0,68–0,75		1,75
Normal			
Kidson			
Askew, Chittenden			
Excess of potash	2,73	< 0,1	
Potash deficient	0,67		
Normal		0,5 –0,63	
Kidson,			
Askew, Chittenden			
Excess of potash	1,72–2,21	0,16–0,37	
Potash deficient			
Normal		0,48–0,63	

the centre of the leaf in most apple varieties. An exception to this is the variety Manks Codlin, the leaves of which show less marked differences between potash and magnesium deficiencies.

A second difference is due to the fact that a magnesium deficiency often shows itself as a chlorosis. Part of the symptoms is a broad yellow zone along the margin with extensions between the veins. In some cases of potash deficiency slight yellowing precedes the death of the margin. Necrosis of the leaf margin caused by a magnesium deficiency (varieties Zigeunerin, Signe Tillisch, Yellow Transparant) does not consist only of the death of tissue parallel to the margin but is characterized by an undulating border with penetrations between the veins. Once more the variety Manks Codlin is an exception since in this variety, the dead margin due to a magnesium deficiency is almost as straight as that in case of a potash deficiency and is hardly to be distinguished as the results of a magnesium deficiency.

Under these circumstances therefore only a third difference is of use for diagnosis. A magnesium deficiency induces casting of the leaves whereas in case of a potash deficiency the leaves remain on the tree. This leaf casting occurs particularly on the long shoots, beginning at the base and steadily progressing towards the top of the shoot during the second half of the summer. Some of the leaves at the base remain on the tree.

A fourth difference useful for diagnostic purposes lies in the growth of the leaves. A potash deficiency induces the formation of small leaves but a magnesium deficiency does not influence leaf development.

There are no characteristic external symptoms on the fruit. Maturation is delayed in magnesium-deficient apples and the fruits become tough. Possibly there is a connection between magnesium deficiency and "bitter pit". As magnesium and phosphate go together inside the plant, a magnesium deficiency might induce a low phosphate content in the apple. Chemical analysis of apples has shown that apples with bitter pit have a low phosphate content and a high ash content (B r o w n 1927). The latter condition is also characteristic of leaves with a magnesium deficiency.

Spray trials with phosphate of potash showed that bitter pit can be diminished in this way.

Very interesting is the relation between the amount of fruit on a tree and the severity of magnesium deficiency symptoms. On Cox's Orange Pippin on type IX we observed that trees with many fruits had less leaf cast then those with only a few fruits. The explanation might be that the fruits act as a water reservoir for the leaves. Leaves suffering from a magnesium deficiency show an increased evaporation and cannot withstand a dry and hot day without getting necrotic spots and falling. If there are sufficient fruits to fill the gap in the water supply to the leaves, the results of a magnesium deficiency are less devastating.

Another relation exists between magnesium deficiency and autumn colours. Autumn colours appear to be dependent upon the nutritional factor which has been limiting the growth during summer. This holds only for nitrogen, phosphorus and magnesium. A potash deficiency does not show itself in the autumn-colouring of the leaves.

If nitrogen nutrition is the limiting factor in the growth of the leaves then the colour will become yellow. If the phosphate supply is lacking then a reddish or purple brown colour will develop. Why phosphate deficiency causes the same colour to develop as an excess of carbohydrates is not as yet clear. Probably the phosphate has a role in the transport of carbohydrates.

If neither nitrogen nor phosphate deficiency determine the leaf colour but magnesium is the limiting factor then the same

symptoms develop in autumn as those which precede the leaf casting in summer, namely yellowing near the margin and necrosis between the veins. In other varieties a magnesium deficiency expresses itself in the formation of yellow and red margins along the leaves.

Research in the Netherlands before 1943. In 1937 the first case of a magnesium deficiency was recorded by Miss Ir. J. ter Kuyle on a sandy soil near Breda. Both in England and in Holland (ter Kuyle), leaf samples of diseased and healthy trees were analysed. Miss ter Kuyle kindly forwarded to me the results.

TABLE III

Magnesium contents of apple leaves (1938) expressed in % Mg of dry matter

Variety	Rootstock	Condition		% Mg		Site
		of the tree	of the leaves	in dry matter		
Cox's O.P.	II	diseased	diseased	0,040	0,055	Leur (N.Br.)
,, ,,	II	,,	healthy	0,091	0,100	,,
,, ,,	Doucin	healthy	,,	0,138	0,147	,,
,, ,,	II	,,	,,	0,457	0,447	Wageningen

The difference between healthy and diseased leaves on the same tree indicates that the healthy leaves contain approximately double the content of Mg.

Healthy leaves from a healthy tree in the same orchard contain approximately three times as much Mg as diseased leaves from a Mg-deficient tree.

The contents of the healthy samples from normal trees in the deficient orchard are also below the border concentration given by Wallace.

Four samples were analysed in England (Long Ashton).

TABLE IV

Composition of apple leaves in September as determined by Wallace

Composition % of dry matter	Cox's O.P.		Yellow Transp.		Normal composition of apple leaves in Sept.
	healthy	diseased	healthy	diseased	
Ash	12,3	12,6	11,9	11,9	7,50
CaO	2,5	2,98	2,45	1,9	1,82
MgO	0,22	0,18	0,30	0,059	0,45
K_2O	3,5	2,91	3,52	3,93	1,92
Na_2O	0,009	0,005	0,01	0,019	—
P_2O_5	0,43	0,55	0,37	0,41	0,39

The sample from Wageningen was obtained from a potash-deficient soil and contains as a result, an abnormally high percentage of magnesium.

The following conclusions can be drawn:

1. The ash content of healthy and necrotic leaves from a deficient orchard is almost double that of leaves from a healthy orchard.
2. The calcium content is also rather high.
3. The magnesium content is low or very low ($^3/_4$ to $^1/_{10}$ of the normal content).
4. The potash level is twice as high as normal.
5. The phosphorus content is a little higher than normal, notwithstanding the low magnesium content.

The analyses made in Wageningen and Long Ashton are compared in the following table.

TABLE V

Magnesium contents of leaves of Cox's O.P. determined in Long Ashton and Wageningen

	% MgO in dry matter	
	Wageningen	Long Ashton
Deficient	0,068–0,167	0,18–0,22
Healthy	0,236–0,763	0,45

The analyses made at Wageningen cover a much wider range than those made at Long Ashton.

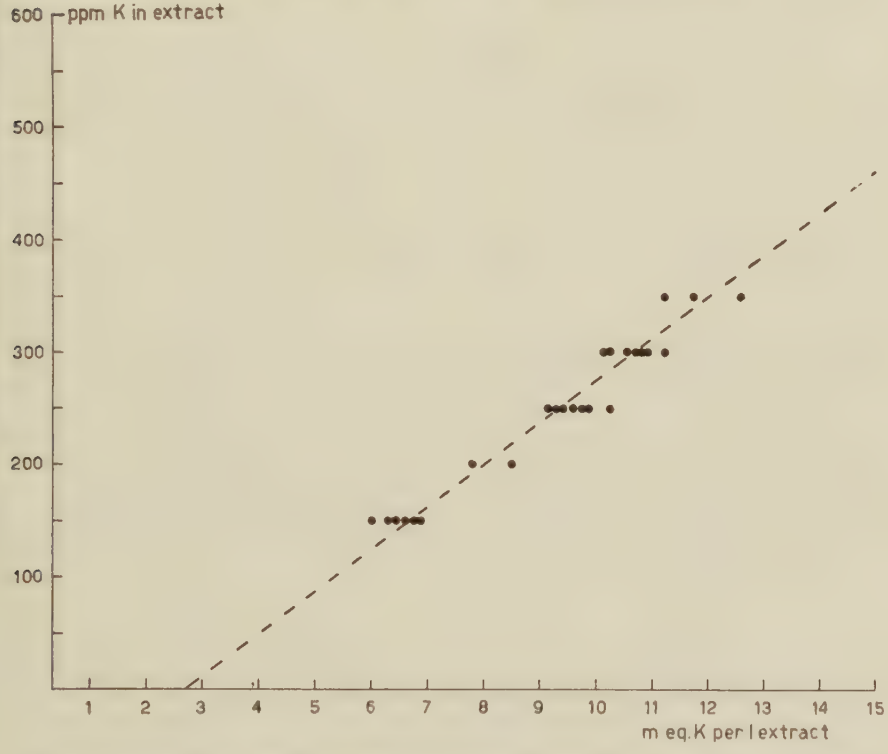
Research in the Netherlands after 1946. Methods*). 1. Potash. The determination of potash was carried out by means of a flame photometer on extracts of dried leaf petioles in 10% H_2SO_4. 0,2 g dried leaf petioles were extracted with 10 cc dilute sulphuric acid.

2. Magnesium. Magnesium was determined by means of the titan yellow method using a photo-electric colorimeter. Instead of analysing extracts, analyses were conducted on the ash of the leaves.

*) A number of potash and magnesium determinations were made in cooperation with the laboratory of the firm Van der Have at Kapelle and the Bureau of Information for Chilean Nitrate. Acknowledgments are due to Mr van der Have and Dr J. J. Lehr for their willingness to carry out these analyses.

In the Laboratory of "Zeelands Proeftuin" magnesium was determined colorimetrically according to the titan yellow method as described by Nicholas. Extracts were made by using Morgan's reagent (sodium acetate and acetic acid); 0,4 g dried and ground leaf petioles were extracted in 10 cc Morgan's reagent.

Using these different methods, very different results for potash and magnesium were obtained, depending on whether the material was extracted or ashed and also on the extracting reagent.



The correlation between the determination of potash in Morgan's extracting reagent by means of the flame photometer and by means of the colorimetric method. Vertically colorimetric determination, horizontally flame photometer method.

Between the flame-photometer potash determination and the colorimetric determination a good correlation could be established Fig. 1.

The flame-photometric method is more sensitive than the colorimetric, with the result that the former still indicates 0,3–0,4 m.eq.K/l when the latter gives zero values.

There is very little agreement between the colorimetric determination of magnesium after extraction and after ashing of the petioles. Four samples were analysed in three ways:

1. colorimetric determination in the extract at Wilhelminadorp
2. ,, ,, ,, ,, ,, ,, Wageningen
3. ,, ,, after ashing ,, ,,

Results were as follows.

TABLE VI

No.	extract *) Wilhelminadorp	extract *) Wageningen	ash **) Wageningen
349	60	10	320
350	40	19	180
351	190	16	1350
352	75	5	290

*) expressed as ppm extracting reagent.
**) ,, ,, ,, dry matter.

There is only slight agreement between extract analyses made in Wilhelminadorp and ash analysis made in Wageningen.

In the extraction method, one determines only that fraction of the magnesium which is dissolved in the vacuole and in the protoplasm. All that is fixed in the chlorophyl cannot be determined in this way. On the other hand with the ash analysis method one determines the total amount present. This difference is shown in table VI.

Results. Material: From four orchards, samples were collected and compared for magnesium and potash content. A number of samples were taken from top and base of the long shoots and from the fruit buds. In each of these orchards a magnesium deficiency was prevalent. For comparison a great number of leaf samples was collected from normal and potash-deficient orchards. The time of picking varied from August to October.

From table VII the following conclusions can be drawn:

1. The magnesium contents of leaves from healthy trees are 4–6 times higher than those from diseased trees (according to ash analyses).

TABLE VII

Magnesium and potash contents of apple leaves from Mg-deficient orchards

Variety	Condition of the tree	Situation of the leaf	% Mg in dry matter	m.eq.K/l in extract	ppm Mg in extract	Condition of the tree	Situation of of the leaf	% Mg in dry matter	m.eq.K/l in extract	ppm Mg in extract
Golden delicious	diseased	top of shoot	0,130	10,70	160	—				
,, ,,	healthy	base of shoot	0,135	8,52	90	diseased	base of shoot	0,066	15,99	50
Laxton's Superb XVI	,,	top ,, ,,	0,196	8,13	190	,,	,, ,, ,,	0,049	10,40	75
Laxton's Superb IX	diseased	,, ,, ,,	0,135	9,86	80	,,	,, ,, ,,	0,029	10,32	30
Cox's Orange Pippin	healthy	base ,, ,,	0,142	10,68	80	,,	,, ,, ,,	0,049	15,75	60
,, ,, ,,	—	—				,,	twigs bearing fruit	0,091	16,60	50
Jonathan IX	healthy	twigs bearing fruit	0,108	8,18	60	,,	base of shoot	0,030	16,60	80
Jonathan XVI	,,	base of shoot	0,191	7,40	120	,,	,, ,, ,,	0,034	10,80	80
Manks Codlin	,,	,, ,, ,,	0,178	6,81	100	,,	twigs bearing fruit	0,083	10,90	100
James Grieve	—	—				,,	midst of shoot	0,032	15,70	60
,, ,,						,,	base ,, ,,	0,018	13,29	40
Zigeunerin	healthy	base of shoot	0,129	12,76	80	,,	,, ,, ,,	0,030	13,54	60
Perzikr. Zomerappel	—	—				,,	,, ,, ,,	0,033	15,45	80

TABLE VIII

Composition of apple leaves in normal, potash-deficient and magnesium-deficient orchards

Normal

Variety	K m.eq/l extr.	Mg extr. ppm
Early Victoria	6,63	80
Manks Codlin	5,96	120
Golden delicious	5,37	120
Goudreinet	5,64	100
Signe Tillisch	7,26	80
Transp. de Cronc.	9,66	120
Notaris	8,32	140
Perzikr. Zomerappel	6,90	140
Witte Wintercalville	8,35	160

Potash deficient

Variety	K m.eq/l extr.	Mg extr. ppm
Cox's Orange Pippin	1,93	200
Cox's Orange Pippin	1,92	250
Cox's Orange Pippin	1,46	170
Laxton's Superb.	1,13	280
Laxton's Superb.	1,68	360
Golden Delicious	2,83	180
Bramley's Seedling	1,12	400
Yellow Transparant	1,42	350
Zigeunerin	1,13	280
Belle de Boskoop	2,23	140
Sterappel	1,50	260

Magnesium deficient

Variety	K m.eq/l extr.	Mg extr. ppm
Cox's Orange Pippin	13,52	60
Cox's Orange Pippin	13,35	60
Cox's Orange Pippin	10,84	100
Golden Delicious	11,68	80
Transp. de Cronc.	18,25	50
Yellow Transparant	9,83	150
Early Victoria	11,52	90
Manks Codlin	9,00	140
Belle de Boskoop	15,36	120
Signe Tillisch.	9,06	100
Groninger Kroon	11,85	30
Groninger Kroon	14,15	80
Witte Wintercalville	11,60	70

2. The potash contents of leaves from diseased trees are 25–50% higher than those from healthy trees (according to extract analyses)

3. Agreement between ash analysis and extract analyses is not always very clear.

4. Basal leaves of healthy trees have only little more extractable magnesium than basal leaves of diseased trees, but in the former case the quantity of magnesium in the ash is much higher.

From the latter statement might be deduced that the magnesium content of the basal leaves from a healthy tree already decreases early in the summer.

In the top leaves from a healthy tree the magnesium content is much higher, as the analysis of the extract shows.

If the top leaves are picked from a diseased tree, the extractable magnesium is at the same level as that in the healthy basal leaves.

By comparing the results of Ir t e r K u y l e with the above figures, one can conclude that the figures for magnesium deficiency are at the same level. Samples with so great an amount of magnesium as those from Wageningen have not been collected in Zeeland.

To obtain a general impression a number of samples from normal and potash deficient orchards was collected. The analyses for potash and magnesium give substantial evidence for the disturbed balance between these two elements in the case of a deficiency of one of them (table VIII).

Control. A spray trial with 2% $MgSO_4.7H_2O$ was carried out in 1947. This salt is the easily soluble Epsom salts containing 51% H_2O. The difficultly soluble Kieserite ($MgSO_4.1H_2O$) is useful for fertilizer trials. The experiment was carried out on three trees of the varieties Zuccamaglio, Jonathan and Cox's Orange Pippin on type IX. The dates of spraying were May 24th, June 9th, June 26th, July 14th. Control of the symptoms of magnesium deficiency was not quite complete, but the difference between the sprayed and unsprayed trees was very apparent. The sprayed trees showed less leaf cast and the abnormal discoloration in the autumn was less severe. The experiment was made in an orchard with a very severe magnesium deficiency where some trees were almost completely defoliated by the end of the summer. It is probably necessary to spray as many as 6 times, or with a higher concentration, in this badly diseased orchard. No spray damage has been observed

despite the fact that the sprays were applied under varying weather conditions. English experiments have shown that even 4% magnesium sulphate can be sprayed without harming the trees.

Soil tests. Determinations of the magnesium and potash content were carried out on soil samples from 20 and 40 cm depths in several orchards. These results are giver in the following table. Some figures for soil analyses carried out at Groningen are added to give some idea of the potash content and percentage of clay particles ($< 2\,\mu$) in these soils.

TABLE IX

Potash and magnesium contents determined by means of the Morgan-Venema method on some clay soils compared with the results of soil analyses from the Soil Analysis Laboratory at Groningen

Condition of the trees	Depth of sample 0–20 cm		Depth of sample 20–40 cm		Analyses at Groningen			
					% K_2O		% clay particles	
	ppm K in soil extract	ppm Mg in soil extract	ppm K in soil extract	ppm Mg in soil extract	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Mg-deficient	100	50	15	50				
,, ,,	100	50	20	50				
,, ,,	130	80	20	50				
Normal	140	180	15	60				
Mg-deficient	110	60	20	40				
Almost normal	130	180	125	140	0,047	0,021	39	29
,, ,,	220	175	200	80	0,058	0,042	29	29
Mg-deficient	50	80	30	140				

Table IX reveals that soils from magnesium-deficient orchards have a rather low content of magnesium and are high in potash in the surface layer.

The figures from Groningen show that the potash content lies above the limit mentioned in the introduction.

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LITERATURE

1) Boynton, D. and Burrell, A. B., Potassium-induced magnesium deficiency in the Mc Intosh apple tree. Soil Sci. **58**, 441–454 (1944).

2) Boynton, D., Studies on the control of magnesium deficiency in New York apple orchards. Proc. Am. Soc. Hort. Sci. **46** (1945).

3) Brown, W., Chemical studies in the physiology of the apple VI. The relation between mineral composition and other characteristics. Ann. Botany 1927.
4. Chucka, J. A., Waring, J. H. and Wijman, O. L., Magnesium deficiency in Maine apple orchards. Proc. Am. Soc. Hort. Sci. **46**, 13–14 (1945).
5) Goodall, D. W. and Gregory, F. G., Chemical composition of plants as an index of their nutritional status. Technical Communication, No. **17**. Imperial Bureau of Horticulture and Plantation Crops, 1947.
6) Kidson, G. B., Askew, H. O. and Chittenden, E., Magnesium deficiency of apples in the Nelson distr. of New Zealand J. Pomology Hort. Sci. **XVIII** 119 (1940).
7) Kidson, E. B., Askew, H. O. and Chittenden, E., Magnesium deficiency of apples in the Nelson distr. New Zealand. New Zealand J. Sci. Techn., **A XXI**, 305–318 (1940).
8 Southwick, L. and Smith, C. F., Further data on correcting magnesium deficiency in apple orchards Proc. Am. Soc. Hort. Sci. **46** (1945). Biol. Abstracts 20 (409).
9. Wallace, T., Chemical investigations relating to magnesium deficiency of fruit trees. J. Pomology Hort. Sci. **XVIII**, 145–160 (1940).
10) Wallace, T., Mg deficiency in fruit trees: the comparative base status of the leaves of apple trees and of gooseberry and blackcurrant bushes receiving various manurial treatments under conditions of magnesium deficiency. J. Pomology Hort. Sci **XVIII**, 261 (1940).

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MANGANESE IN RELATION TO PHOTOSYNTHESIS

II. REDOX POTENTIALS OF ILLUMINATED CRUDE CHLOROPLAST SUSPENSIONS

by F. C. GERRETSEN

Agricultural Experiment Station and Institute for Soil Research, T.N.O. Groningen

Introduction. The author has previously shown [10]) that manganese deficiency in oats is accompanied by a reduced rate of photosynthesis and the question is consequently raised as to the point at which manganese becomes involved in this complicated sequence of reactions in the plant.

Various authors [22]) [24]) [25]) [26]) have pointed to the possibility that manganese and iron together "are mutually coordinating catalysts for oxidation and reduction reactions". Definite proof of the specific role played by manganese in these processes is, however, still lacking.

Redox potential measurements seemed to be the most promising method for elucidation of the problem. Since it would be rather difficult, if not impossible, to use intact leaves for this purpose, and the results of some preliminary experiments using suspensions of intact green algal cells (*Hormidium nitens*) were not encouraging, it was decided to use crude chloroplast suspensions obtained from crushed *Avena* leaves. Aronoff [2]) is the only author who has investigated the redox potentials of chloroplast suspensions; he used various quinones at stabilized redox potentials and showed that the rate of evolution of oxygen depends on the redox potential of the compound used. As it was the aim of the present investigation to ascertain the influence of illumination on redox potentials the latter had to be determined electrometrically. Washed and centrifuged chloroplasts, as used by Warburg [30]) and others [7]) [12]) [14]), seemed unsuitable for the purpose since constituents playing an important role in oxidation reduction processes may be removed by such processes. The experiments of French *et al.* [7]), who showed that the activity of

chloroplasts washed with water and centrifuged could be increased 43% by adding boiled aqueous leaf extract, distinctly point to this. Rabinowitch[19]) p. 355) is of the opinion that the inability of isolated chloroplasts to carry on photosynthesis is an indication that this process requires the participation of the cytoplasm.

The use of an isotonic sugar solution, as used by Ewart[5]), Hill[12]) and others, seemed inadvisable in this case as sugar, being a potent hydrogen donator, might easily upset the whole course of the oxidation reduction reactions. Although in a number of cases buffer solutions containing potasium phosphate or sodium bicarbonate were used, in some experiments suspensions of chloroplasts in water were used. Due to the presence of cell constituents such suspensions had a certain amount of inherent buffer capacity and in addition the possible inactivation of minute traces of heavy metals, due to precipitation by an excess of phosphate ions, was avoided. Moreover any pH changes in the suspension would be more pronounced under such conditions; this proved to be of great importance during the course of the experiments.

Illumination of such chloroplast suspensions generaly brought about large and rapid changes of the redox potential. Although the author is aware that the greatest care must be taken in drawing conclusions from results obtained with such disorganised and heterogeneous systems, the cause of these potential changes could be traced in a number of cases and valuable information was acquired concerning the nature of oxidation reduction processes which may occur in the plant.

Generally the changes in potential were accompanied by small, but very distinct and characteristic, changes of pH; this necessitated a careful watch being kept on pH changes during the experiments. A detailed account of pH changes during the experiments will be given in a subsequent paper.

Experiments with chloroplast suspensions from various plant species *) showed that with species other than *Avena sativa* the potential changes were generally much smaller and less characteristic and

*) Chloroplast suspensions from the leaves of the following plants were used: *Triticum vulgare*, *Triticum cereale*, *Triticum repens*, *Vicia faba*, *Lycopersicum esculentum*, *Solanum tuberosum*, *Urtica dioeca*, *Ranunculus ficaria*, *Aesculus hippocastanum*, *Vallisneris spiralis*, *Trifolium pratense*, *Beta vulgaris*, *Raphanus sativus*.

therefore subsequent investigations were limited to the chloroplasts of *Avena sativa*.

The observations of K u m m and F r e n c h [14]) should be mentioned in this connection. They investigated the evolution of oxygen from chloroplast suspensions and found that, of 28 different species, the chloroplasts of only 3 species showed satisfactory activity; they also found that comparable preparations from the same species could give as wide a range of activities as preparations from different species.

As the time which could be spent on these investigations was limited, the experimental plant material had to be produced through out the year and in winter artificial illumination was used. It could not be avoided therefore that the material used in these experiments was less uniform than might have been desirable.

Experimental methods. Leaves of *Avena sativa* grown, with or without added manganese, in water culture or soil were used as experimental material. Material grown under conditions of artificial illumination in winter appeared to give more pronounced changes of potential than material produced during the normal growing season.

Chloroplast suspensions were prepared by grinding half a gram of fresh young leaf material with 20 ml of water and 1 or 2 grams of quartz sand in a mortar followed by filtration through a very fine mesh silk cloth. The filtrate was centrifuged for one minute at a speed of about 2000 revs.per minute to remove sand and cell wall material. The resulting suspension contains intact and fragmented chloroplasts as well as grana, stroma and cytoplasm. The absence of intact cells was proved by microscopic examination. In some cases 0.01 Mol. phosphate buffer solution (pH 6.5) or a 0.01-0.005 N solution of sodium bicarbonate was used instead of water.

8 ml. of chloroplast suspension were introduced into a glass cell (fig. 1) containing a bright platinum or gold electrode, a glass electrode, a calomel half cell, gas inlet and outlet tubes and a tube for the introduction of materials whose effect on the redox potential was to be observed. In those cases where it was desired to keep the chloroplast suspension in close contact with air or oxygen a flat type of glass cell was used.

Special attention has been given to the electrical apparatus used in the determination of redox potentials and the potential of the glass electrode. With this in mind an electrometer triode (Osram

type T) was used in conjunction with a calibrated potentiometer and sensitive galvanometer; this made it possible to determine potentials to one tenth of a millivolt. The redox potentials are calculated with reference to the normal hydrogen electrode. The greatest care has to be taken to ensure that a grid current does not flow in the triode as this would cause polarisation of the electrodes and thereby introduce severe errors in the potential readings.

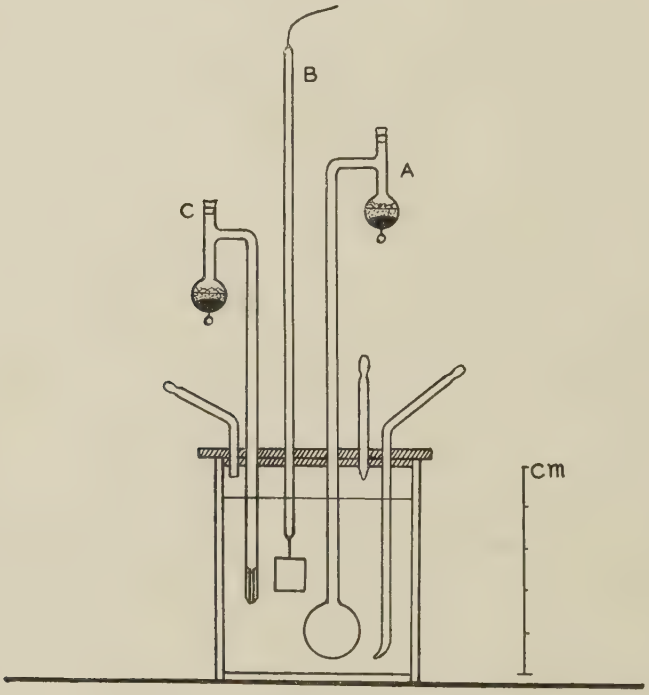

Fig. 1. Cell used for measurement of pH and redox potential of chloroplast suspensions.

(*a*) Glass electrode with calomel electrode.
(*b*) Pt or Au electrode.
(*c*) Saturated calomel half cell.

The cell containing the electrodes and chloroplast suspension was almost submerged in a thermostatically controlled water bath. It proved necessary to disconnect the thermostat from the mains whilst potential readings were being made as the switching of the thermostat disturbed the potential of the glass electrode. It was also found

necessary to screen the connection between the glass electrode and the electrometer. A carbon arc lamp (Zeiss, 4 Amp.) having a horizontal anode was used as a light source and the light passed through two condenser lenses and a water filter in order to absorb ultra violet and heat rays. The illumination amounted to approximately 45,000 lux (fig. 2).

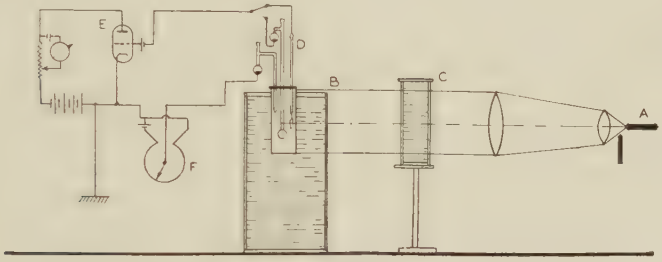

Fig. 2. General layout of the apparatus.
(*a*) Carbon arc lamps (Zeiss 4 Amp.).
(*b*) Constant temperature water bath.
(*c*) Glass cell for absorption of heat rays.
(*d*) Electrode vessel.
(*e*) Electrometer triode.
(*f*) Potentiometer.

Redox potential measurements.

Material used: 500 mg from second leaf below apex of a healthy, outdoor grown, 3 month old (15/3–15/6) oat plant in 20 ml. 0.005 N sodium bicarbonate buffer solution. Temperature 20°C and initial pH 6.94.

The graph (fig. 3) shows a small decrease in the redox potential *) whilst the chloroplast suspension is in the dark before the light is switched on; there is also a similar decrease in pH caused by the carbon dioxyde produced by residual respiration of the suspension. After the light is switched on there is a short induction period which is followed by a rapid increase in potential amounting to 130 mV. The redox potential decreases immediately on switching off the light, rapidly at first then gradually slowing down. On illuminating the suspension for a second time the potential immediately increases

*) An increase (decrease) in potential indicates a shifting of the redox equilibrium towards a more oxidised (reduced) stage. The sign of the potential is the same as the sign of the electrode which is in accordance with the European system and contrary to American practice.

again until a maximum, of about the same level as attained during the first illumination, is reached.

The corresponding pH changes are characterised by an initial sharp increase from the commencement of the first illumination period, indicating that the suspension has become slightly more alkaline, followed by a fairly rapid decrease in pH which continues throughout the experiment the suspension becoming more acid most probably as a result of organic material being photo-oxidised to carbon dioxide.

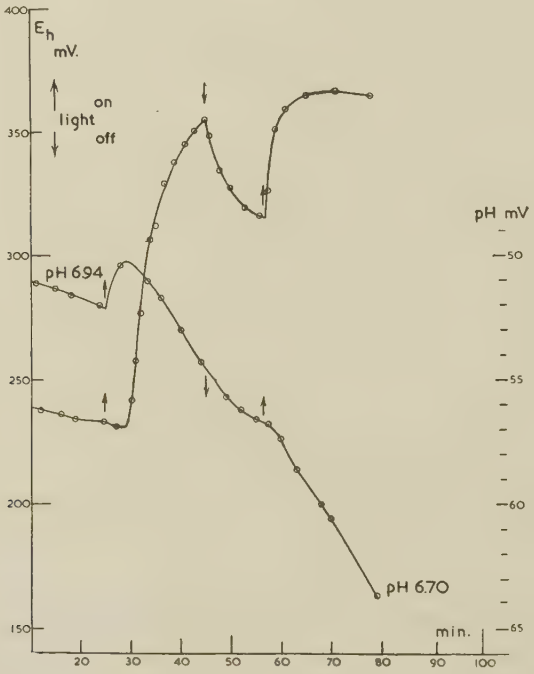

Fig. 3. General course of the changes in redox potential and pH of an illuminated normal crude chloroplast suspension. The rapid increase of potential on illumination and decrease when the suspension is placed in the dark should be noted.

There is a short induction period at the beginning of the first period of illumination which is absent in the second.

The following points arising from the results of the above experiment may be emphasized. At the commencement of the first illumination period there is a slight decrease in redox potential and it is

five minutes before the potential begins to increase. Several investigators [13] [15], using intact leaves or cells, have observed induction periods of two to five minutes from the beginning of illumination and in this respect the crude chloroplast suspensions behave in a similar manner to the intact cells.

It should also be noted that a second lag period was not found to be associated with the commencement of the second illumination period, the potential increasing from the beginning of the illumination in this case. The absence of a second induction period after previous illuminations has also been observed by Gaffron [8] during his experiments on photosynthesis in an atmosphere of hydrogen.

Although the pH changes will be discussed in detail later, attention is drawn to the fact that at the commencement of the second illumination period there is only a slight indication of a change in rate of acidification whereas there was a distinct increase of pH at the beginning of the first period of illumination.

In one exceptional case far more pronounced changes in redox potential were observed (fig. 4).

Material used. 700 mg from second-leaf of a healthy oat plant, grown in the greenhouse under conditions of artificial illumination between September and December, in 25 ml of water. Temperature 22°C. Gold electrode. pH at end of experiment 6.2 (fig. 4).

In this experiment also there is a slight decrease of potential in the dark and a short induction period at the beginning of the illumination period which is followed by an extremely rapid increase of potential of 225 mV to a potential of 516 mV at C when, *although the illumination is continued*, the potential suddenly decreases as rapidly as it increased at the beginning. The illumination was switched off at D and during the short dark period DE the potential decreased much more slowly than in the light but much more quickly than in the initial dark period AB at the beginning of the experiment. When the illumination was switched on again at E the potential continued to decrease at the same rate as before the dark period DE. The introduction of air at F caused an immediate reversal of the decreasing potential which increased to an even higher level (534 mV) than that reached in the first illumination period. Although illumination was continued the potential again decreased after point G was reached

and the introduction of a further quantity of air at *H* produced the same effect as at *F*.

The most important result from this experiment is the attainment of a potential greater than 500 mV which is extremely high for bio-

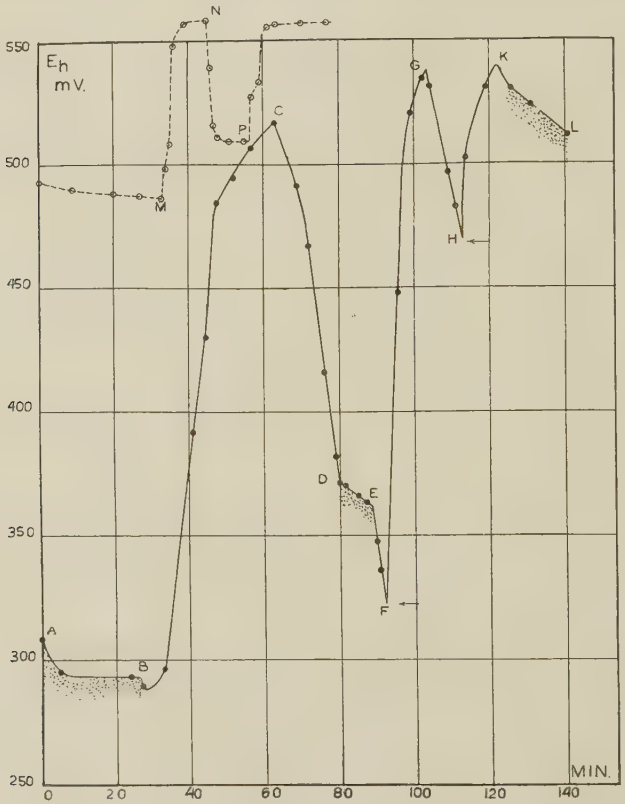

Fig. 4. Exceptionally high potentials observed during illumination of an apparently normal suspension. The sudden reversals in change of potential at C and G, caused by lack of oxygen, should be noted. At F and H air has been introduced. Black dots indicate that the suspension is in darkness. Dotted line indicates the potentials of a dilute solution of hydrogen peroxide; a small amount of chloroplast suspension was added to the solution at M and at N the light was turned on; at P the light was turned off. The height of the potential is the same as that of the original chloroplast suspension.

logical material. Similar high potentials have also been observed in aerated cultures of pneumococci and some lactic acid bacteria. According to Hewitt[11]) these bacteria, which do not contain

catalase, have redox potentials varying from 420 to 510 mV according to the amount of hydrogen peroxide produced. On measuring the potential of a 0.3% solution of hydrogen peroxide in phosphate buffer at pH 6.9 a value of 490 mV was obtained whilst Hewitt recorded a potential of 520 mV with a 0.02% solution of hydrogen peroxide in saline. On adding a suspension of crushed leaf to the 0.3% solution of hydrogen peroxide in phosphate buffer the potential increased from 490 mV to 558 mV accompanied by vigorous evolution of oxygen. Illumination reduced the potential to 510 mV which is exactly the same potential as obtained with the original chloroplast suspension (see dotted line in fig. 4).

This similarity of behaviour points for the possibility that hydrogen peroxide has also been formed during illumination of the chloroplast suspensions. Ultra violet light, which might have been responsible for the formation of traces of hydrogen peroxide, was prevented from reaching the experimental material by the various filters and lenses through which the light passed.

The introduction of pure oxygen into the suspension, whilst it is in darkness before illumination has taken place, has no appreciable effect on the potential causing an increase of not more than 10–20 mV; therefore the extremely high potentials reached cannot be attributed to oxygen production.

As the experiments of Hill [12]) and the others have shown that chloroplast suspensions retain their ability to decompose water there seems to be no objection to the assumption that the crude chloroplast suspensions used in the present experiments also possessed very powerful photosensitive reduction and oxidation systems.

By analogy with the previously mentioned experiments on bacterial cultures oxygen could serve as a hydrogen acceptor and become reduced to hydrogen peroxide. As soon as the supply of oxygen is exhausted the formation of hydrogen peroxide not only stops but the hydrogen peroxide already formed itself acts as a hydrogen acceptor and quickly disappears as it is reduced to water. This explains the potential decreases at *C*, *G* and *K* during illumination.

When further oxygen is introduced it immediately acts as a hydrogen acceptor with the result that hydrogen peroxide is again formed; this is accompanied by a corresponding rise of potential as is observed at *F* and *H*.

The very sharp peaks in the curves at *C* and *G* indicate that the

reduction processes proceed with great intensity; this is the reason why the hydrogen peroxide would be detectable, in spite of the presence of catalase in the suspension, since the velocity of the hydrogenation reaction would far exceed that of the enzymatic decomposition of hydrogen peroxide.

The slope of the curve from *D* to *E* and *K* to *L* during the dark periods is much steeper than the corresponding part of the curve from *A* to *B* before the suspension has received any illumination. This may indicate a stronger tendency towards reduction caused by the persistence of an intermediate reducing compound formed during illumination. This suggestion is strongly supported by the experiments reported on page 170 and 173).

Although the formation of hydrogen peroxide would provide a possible explanation of the observed phenomena in the above experiments it must be emphasized that such observations cannot be regarded as proof for the proposal that hydrogen peroxide is an intermediate in normal photosynthesis nor do they exclude the possibility of the formation of other peroxides, metal or organic.

The effect of Manganese on Redox Potential. Although it was only in one case out of a hundred experiments that, in an apparently normal chloroplast suspension, a potential of more than 500 mV was observed it seemed that this one case was of sufficient importance to try and ascertain the cause of the interesting phenomenon. Generally the increase of potential on illumination did not exceed 100 mV; this was not caused by lack of oxygen as neither introduction of air nor bubbling pure oxygen through the suspension had any appreciable effect on the potential.

During the course of the experiments it was shown that the addition of traces of manganese as manganous sulphate, although it did not affect the redox potential of the chloroplast suspension whilst it was in darkness, caused the potential to increase in many cases to about 500 mV or even higher as soon as the suspension was illuminated.

Experimental Material. 700 mg from two young leaves of a healthy oat plant with slightly chlorotic leaves, grown under conditions of artificial illumination in the greenhouse between September and December, in 25 ml water. Temperature 21°C. pH 6.55. Gold electrode.

In fig. 5 the changes in the redox potentials of suspensions from two identical leaves of the same oat plant are plotted against time in minutes. The potential of the suspension from the first leaf was

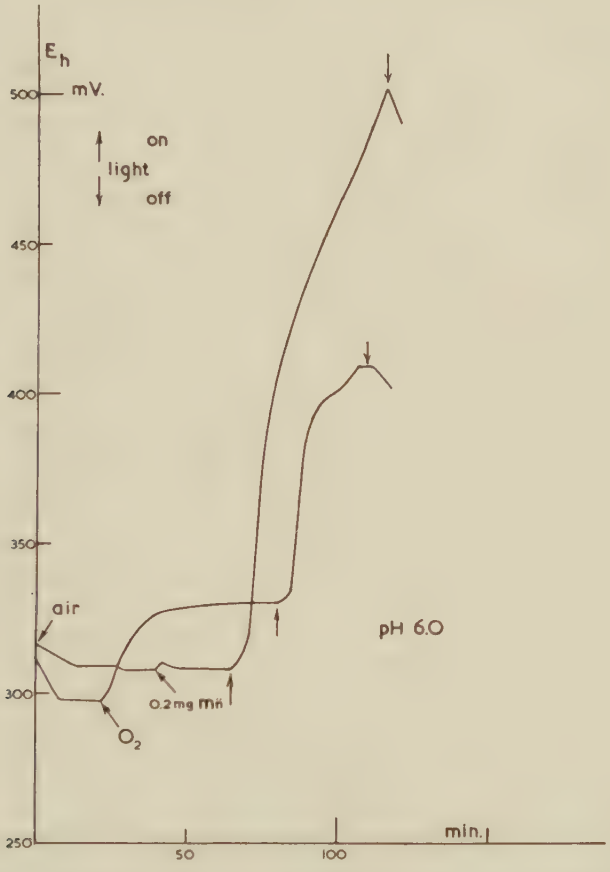

Fig. 5. Redox potential curves of suspensions from two identical leaves of the same oat plant.

I: Oxygen is not the direct cause of the high potentials since introduction of this gas only causes an insignificant increase of potential.

II: Addition of manganous sulphate whilst the suspension is in the dark has no effect on the potential; subsequent illumination, however, results in a rapid increase of potential to 500 mV thereby indicating that peroxide has been formed.

initially 310 mV and thereafter decreased to 300 mV and remained fairly constant whilst in the dark. On bubbling oxygen through this

suspension the potential increased by 25 mV after which it remained constant again. When the suspension was now illuminated the potential increased by 75 mV to 400 mV.

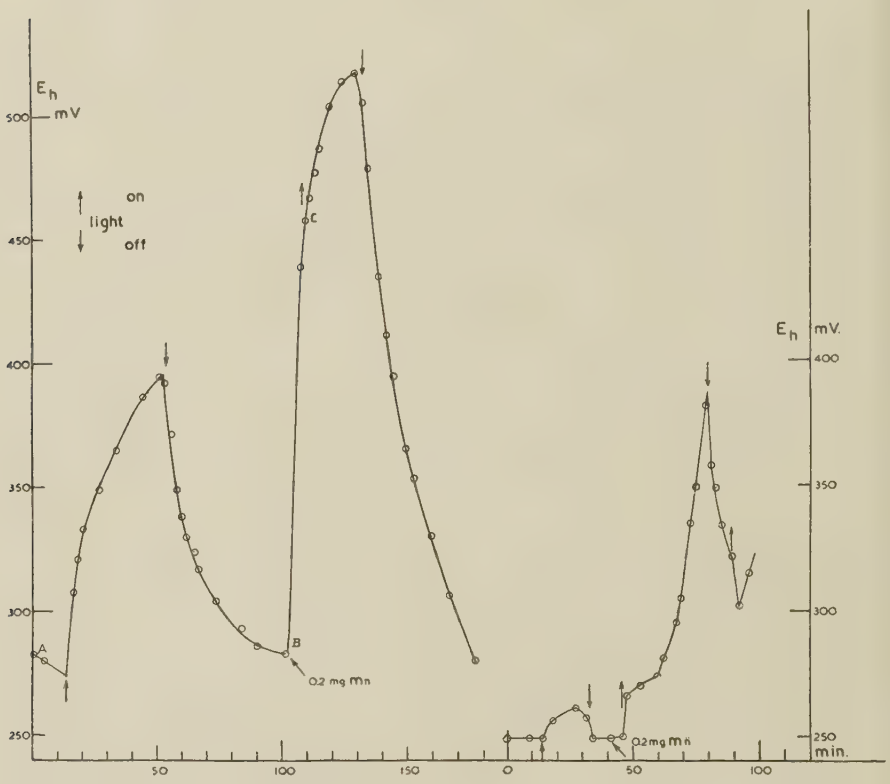

Fig. 6. Redox potential curves of suspensions from similar leaves from two oat plants of the same age, the curve on the left is that of a suspension from a plant grown in presence of manganese and that of the right of a suspension from a plant highly deficient in manganese.

Curve on left of Fig. At the commencement the redox potential changes have the same courses as in Fig. 3. The addition of 0.2 mg Mn^{++} as $MnSO_4$ at B *whilst the suspension is in the dark*, after previous illumination causes the potential to increase to more than 500 mV.

Curve on right of Fig. At the commencement hardly any change is produced in the potential by illumination of the suspension and in this case there is very little change in the potential on addition of manganese whilst the suspension is in the dark; when the suspension is now illuminated there is a rapid increase of potential indicating that reactivity is restored.

The potential of the suspension from the second leaf remained fairly constant whilst kept in the dark and the addition of 0.2 mg of manganese as manganous sulphate did not alter the potential appreciably. When this suspension was now illuminated the potential increased to 500 mV indicating that peroxide had been formed.

In the following experiment (fig. 6) two leaves from different oat plants of the same age were compared, the first a healthy leaf from a normal plant (the same material as used in the previous experiment) and the second an etiolated leaf from a plant of the same age but deficient in manganese.

The suspension from the healthy leaf showed the same potential changes with time, before the manganese was added, as similar suspensions in previous experiments; after addition of manganese the potential increased to 516 mV.

The curve of potential changes in the chloroplast suspension from the etiolated, manganese deficient, leaf shows quite a different picture. When the light is switched on, 15 mins. after the commencement of the experiment, the increase in potential amounts to only 12 mV which is only one tenth of the increase observed in the suspension from a normal leaf. If the suspension is now placed in the dark, addition of manganese sulphate does not cause any change in potential but when the suspension is now illuminated the redox potential increases by nearly 150 mV. The fact that in the manganese

TABLE I

The influence of Mn^{++} on the redox potentials of different crude chloroplast suspensions

Nr. and date of Expt.	I 28.4	II 19.4	III 26.4	IV 27.4	V 28.4	VI 15.10	VII 26.11	VIII 1.12	IX 11.12	X 12.12
Initial pot. dark . . .	243 m.V.	284	298†)	315	239	283	258	357	294†)	270†)
Max. pot. after illumination, without Mn .	379	248	405	—	—	395	311	312	389	378
Pot. directly after addition of 0.2 mg Mn to 8.5 ml suspension *) .	380	253	384	309	256	283	304	310	382	372
Max. pot. + Mn, in light and with O_2 or air ..	454	471	502	502	495	516	479	461	586	590
Total rise of potential after addition of Mn^{++} m.V.	66	218	118	194	235	233	175	151	204	228

*) $MnSO_4$ added in the dark.

†) measured with gold-electrode.

deficient leaf the lack of reactivity can be restored immediately by the addition of a trace of manganese makes it highly probable that the function of manganese in the normal leaf is of the same nature as made itself apparent in these crude chloroplast suspensions.

The mean value of the maximum potentials reached after the addition of manganese was 505 mV which is 153 mV higher than in the absence of manganese: the most likely explanation of these high potentials is that they indicate the formation of hydrogen peroxide by photohydrogenation of oxygen, this reaction being catalysed by the presence of manganese. (See table I).

There are a few points which seem to be of special interest in this connection. Whilst the suspension is in the dark before it has been illuminated the potential changes are rather small and of little importance. When, however, the suspension is in the dark after being illuminated the potential which has been reached in the light is not maintained but decreases very rapidly in a number of cases (see fig. 6, left hand curve). This may be due to the decomposition of hydrogen peroxide by catalase or to the suspension retaining its reducing capacity for some time after being placed in the dark. The latter supposition seems to be more likely for when manganese is added to such a suspension, which is *in the dark* after previous illumination, the potential increases by more than 200 mV in a few minutes. This indicates that the suspension at *B*, which has been illuminated previously, has a much greater reducing capacity than the original suspension at *A* (notwithstanding the fact that both the points *A* and *B* have the same redox potential) and to such an extent that the formation of hydrogen peroxide in the dark is possible. Under these circumstances further illumination at *C* does not even change the rate at which the potential increases thereby indicating that the residual reducing compound remaining from the previous illumination period was sufficient to hydrogenate the oxygen dissolved in the suspension.

This explanation of the experimental observations is confirmed by the results of the next experiment (fig. 7).

Experimental Material used: 500 mg from leaf of oat plant, grown in the greenhouse under conditions of artificial illumination for three months between 15/9–12/12, in 20 ml. 0.005 N. sodium bicarbonate buffer. Temperature 20°C. Platinum electrode. pH 7.17.

At the beginning of the experiment pure oxygen was bubbled through the suspension for a few minutes; this procedure also replaced

Fig. 7. Curve of redox potential of chloroplast suspension showing how the introduction of oxygen still causes a very rapid increase of potential although the suspension has been in the dark for 20–40 minutes following a period of illumination. Note the difference between the equipotential points A and B; both occur whilst the suspension is in the dark, A before the suspension has been illuminated at all and B whilst the suspension is also in the dark 20 minutes after the end of the first illumination period.

the air above the suspension with oxygen. As had been repeatedly observed previously, addition of 0.1 mg of manganese at this stage does not cause any appreciable change in the potential. As soon as the suspension is illuminated the potential increases rapidly (26.7 mV per min.) until the oxygen is exhausted when it immediately begins to decrease and ultimately reaches a minimum of 52 mV. When the light is now switched off the potential increases somewhat and tends to reach a maximum of about 100 mV. This change which takes place over a period of about 20 mins. is not a simple photoelectric effect, which would be an instantaneous phenomenon, but might be regarded as a movement of the redox equilibrium towards a somewhat less reduced state due to the cessation of production of hydrogen atoms.

The introduction of oxygen, whilst the suspension is still in the dark, caused the potential to increase by 260 mV in a few minutes: during the first two minutes the potential increases at not less than 55 mV per min. and very gradually slows down to a rate of increase of 2–3 mV per min. after 20 mins. This diminution in the rate of increase is not brought about by lack of oxygen since when the illumination is switched on again at *C* there is a sudden increase in the potential which is at least six times as rapid as in the previous dark period. It is tempting to suppose that in this case the reserve of reducing substance is finally becoming exhausted and this results in the diminishing rate of increase of potential just before the illumination is switched on at *C*.

It is interesting to note that the reducing capacity of the reducing substance may persist for a considerable time; in the present case it lasted for 45 minutes whilst in another case the addition of manganous sulphate still caused an increase in the potential of 180 mV 115 minutes after the suspension had been placed in the dark and whilst it was still in the dark. A systematic investigation of the various factors affecting the life period of this intermediary substance would appear to be worth while.

The influence of iron on the redox potentials of chloroplast suspensions. The relationships between manganese and iron in plant physiological processes are repeatedly stressed in the literature and it therefore seemed essential to investigate the influence of iron on the oxidation reduction potentials of the crude chloroplast suspensions as well.

Experimental material used. 500 mg of leaf of oat plant, grown in the greenhouse under conditions of artificial illumination for three months between September–December, in 20 ml 0.01 N. sodium bicarbonate buffer solution. Temperature 20°C. Platinum electrode pH 7.1. Oxygen atmosphere above the suspension (see fig. 8).

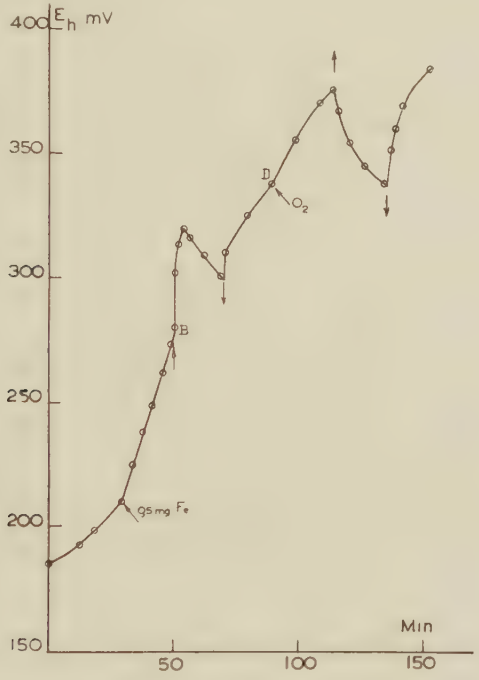

Fig. 8. Curve of potential changes in a chloroplast suspension, having a low initial potential, on addition of 0.5 mg. Fe^{++} as ferrous sulphate. At first there is a period of oxidation which is increased by illumination. When a certain level of potential has been reached during this period of illumination a period of reduction supervenes even in the presence of oxygen, but oxidation predominates when the suspension is placed in the dark.

On adding 0.5 mg iron as ferrous sulphate to 8.5 ml of a chloroplast suspension having an initial potential of 185 mV there was a gradual increase in potential which was considerably enhanced on illumination which commenced at point *B*. Whilst the suspension was being illuminated the curve showed a sudden reversal indicating that photoreduction was greater than oxidation. As soon as the illumination was switched off oxidation became greater than reduction; the oxygen diffusing into the suspension apparently being sufficient

to bring this about since the introduction of oxygen at *D* had no effect on the potential. Although a slow stream of oxygen was constantly passed through the suspension there was a rapid decrease in the potential as soon as the illumination was switched on again and a rapid increase after it was switched off. Thus the suspension behaves differently in the presence of iron and manganese. Under similar conditions manganese caused the potential to increase to 500 mV and even higher whilst iron appeared to bring about a decrease in the potential during continued illumination, after a certain level of po-

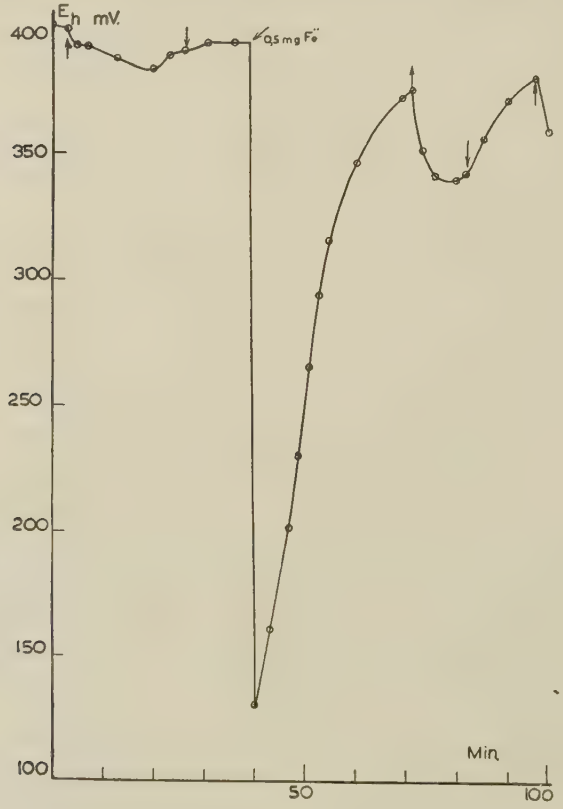

Fig. 9. Curve showing the effect of the addition of 0.5 mg of Fe^{++} as ferrous sulphate on the potential of a chloroplast suspension having a high initial potential. At first there is a sharp decrease, brought about by the excess of Fe^{++} ions, which is followed by rapid oxidation. Subsequent potential changes are similar to those in Fig. 8. reduction predominates on illumination and oxidation while the suspension is in the dark.

tential had been reached. This is confirmed by the next experiment.

Experimental Material used. 500 mg from second leaf of a yellow green oat plant, grown in culture solution deficient in iron from April to July, in 20 ml of buffer solution. Temperature 24°C. Platinum electrode. pH 6.3 (see fig. 9).

The initial potential of the suspension in this case was high being 397 mV. The potential was changed very little by either illumination or darkness. On adding the same amount of ferrous iron to this suspension in the dark an instantaneous decrease in the potential resulted due to the high concentration of ferrous ions compared with ferric ions. Immediately the minimum potential of 127 mV was reached oxidation commenced and proceeded so rapidly that one is forced to assume that this oxidation was accellerated by enzymes or oxidizing complexes formed during the previous illumination period.

Within one hour the original E_h was again reached and when the suspension was now illuminated the potential decreased thereby indicating that photoreduction processes prevailed. After fifteen minutes the potential became constant thereby indicating that at this potential photoreduction and reoxidation, due to the diffusing oxygen, balanced each other. Consequently when the illumination was turned off the potential increased again.

The difference between this and the previous experiment is that in this case the potential decreased after the addition of ferrous iron whereas in the previous experiment it increased. This is made understandable by the fact that the initial potential in the second experiment was nearly 200 mV higher than in the first. In the first experiment the initial potential was quite near the minimum potential which would be reached when such a quantity of ferrous iron is added to the suspension and it is evident that oxidation began immediately.

That the addition of ferric ions, as ferric ammonium sulphate, to a chloroplast suspension in the dark, and having an initial potential of 330 mV, does not influence the redox potential appreciably is shown by the following diagram (fig. 10).

Experimental Material used. 550 mg of second leaf from top from healthy oat plant, grown in complete culture solution under conditions of artificial illumination between October–January, in 20 ml water. 9 ml suspension placed in the cell at a temperature of 20°C. Platinum elec-

trode. pH 7.06. 0.2 mg of ferric iron added as ferric ammonium sulphate in 1 ml of solution. Atmosphere above the suspension air.

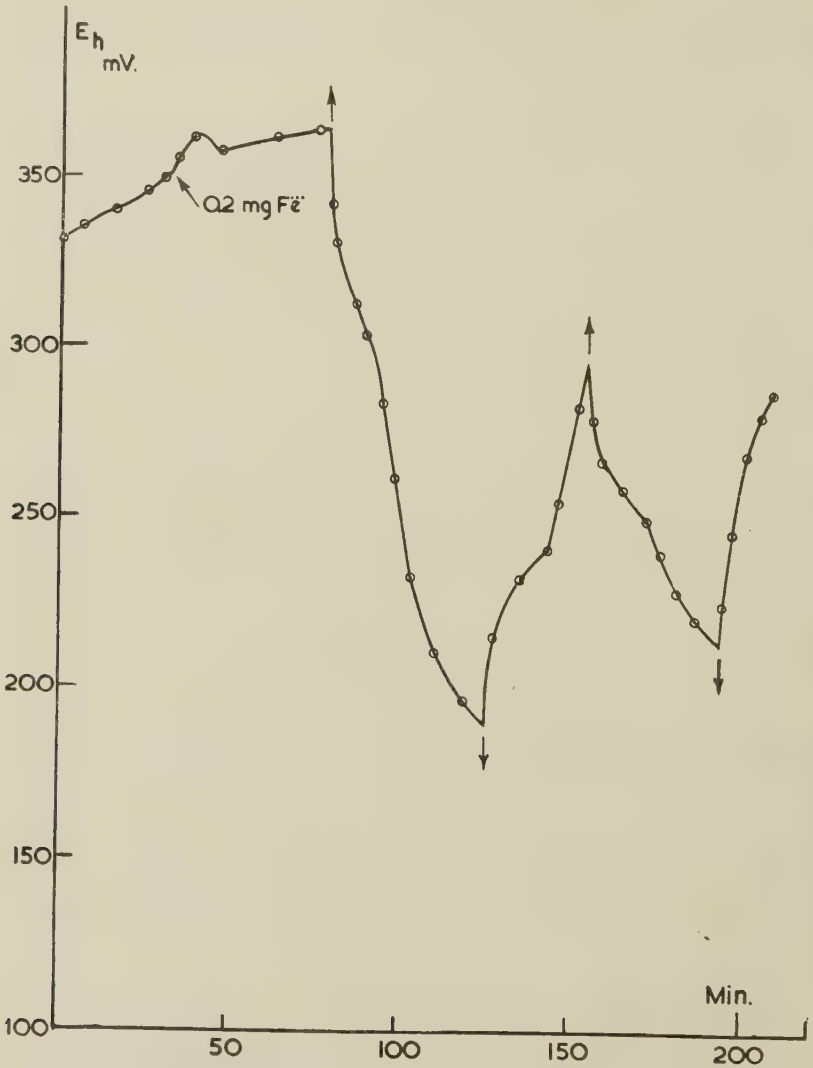

Fig. 10. Curve showing the effect of the addition of 0.2 mg Fe^{+++}, as Ferric ammonium sulphate on the potential of a chloroplast suspension. There is very little effect on the potential whilst the suspension is in the dark at the beginning of the experiment. Illumination of the suspension now produced rapid reduction which is followed by rapid oxidation in the second and third dark periods.

Whilst the suspension was initially kept in the dark the potential increased slowly and the addition of the ferric ions at this stage did not influence the ultimate redox potential reached. When the system was now illuminated the reduction processes were intensified and when a period in the dark followed the illumination the potential increased; the course of the changes in potential followed the same trends as in previous experiments.

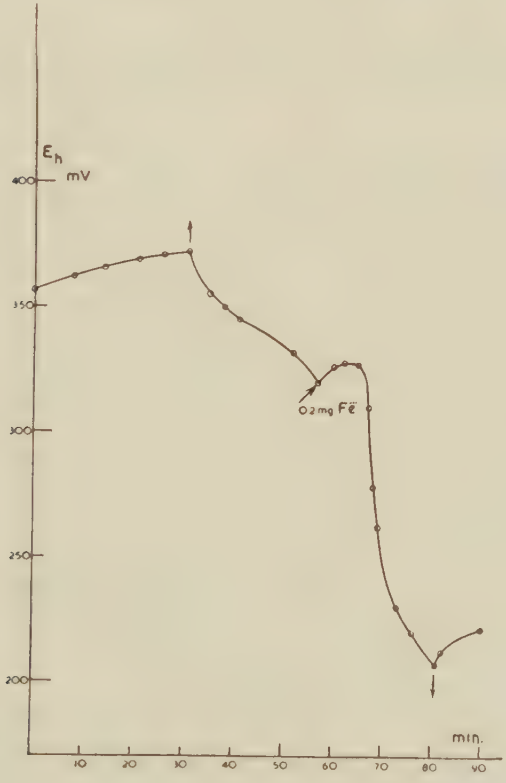

Fig. 11. Curve showing the effect of addition of Fe^{+++} to an illuminated chloroplast suspension on the potential of the suspension. After a small increase in potential due to slight acidification the rate of reduction increases by 4 times.

If a similar amount of ferric ions as ferric ammonium sulfate were added during illumination, using the same material as in the previous experiment, (see fig. 11) there was at first a small increase in potential due to the acid reaction of the salt solution this was followed

immediately by a rapid decrease of the potential. The velocity with which the potential decreased after the addition of ferric ions was nearly four times that in the previous stage in the absence of ferric ions.

On comparing the results obtained by addition of ferrous or ferric ions to the suspension with those obtained by addition of manganous ions there are certain resemblances as well as fundamental differences.

Both elements have a decided influence on the oxidation reduction processes of the suspension whilst it is in the dark as well as during illumination.

At the levels of redox potential which are generally observed with these crude chloroplast suspension the manganous and ferric compounds may be present simultaneously without altering the potential appreciably whilst the suspension is in the dark.

During illumination of the suspension however their activities diverge, the manganous ions showing a strong tendency to be associated with the formation of peroxides and correspondingly high potentials whilst the ferric ions appear to be associated with an increase in the reducing capacity of the suspension and a corresponding decrease of redox potential.

It is interesting to note that these experiments although carried out from a completely different point of view to those of Shive [22]) and Soomers and Shive [24]) point to the same conclusions. Soomers and Shive concluded that the activities of iron and manganese are interdependent and showed that the dry weight of their plants was related to the iron-manganese ratio and not to the absolue concentrations of these elements.

It appears from the present experiments that iron and manganese should be regarded as complementary oxidation reduction agents and that an excess of one element over the other is likely to disturb the oxidation reduction equilibrium in the plant.

Discussion. There seems to be no objection to the supposition that these crude chloroplast suspensions continue to behave as very active photo-oxidation reduction systems which are still capable of decomposing water into its constituents as van Niel and others [18]) have postulated as the first step in photosynthesis.

According to Weiss [35]) [34]) the constituents of water, H and OH,

are free H atoms and free OH radicals *) which are in an extremely reactive state, the OH being a strong oxidizing agent and the H a very active reducing agent. Since the life of these radicals is rather short, of the order of 0.01 to 0.1 sec. it seems logical to assume that the back reaction H + OH = H_2O is in some way prevented, most probably by adsorption of the radicals on to some organic or inorganic acceptor thereby forming intermediate radicals of greater stability which however have retained the greater part of their reactivity. Rabinowitch[19]) in his various schemes for the primary process in photosynthesis, postulates the existence of one or more intermediates between the H atoms and the final reduction process in which CO_2 is involved.

These intermediates may be symbolized as HX or H_2X for the H atoms and it seems logical to assume the existence of similar acceptors in the case of hydroxyl radicals and oxygen atoms as well, giving OZ or OHZ, although they are omitted from the above mentioned schemes for the sake of simplicity.

The fact that in the experiments recorded on pages 170 and 173, chloroplast suspensions are still able to form peroxide, when oxygen or manganous sulphate are added, after 45 to 115 minutes in the dark subsequent to a period of illumination shows that the reducing capacity can be retained during that period of time. This is strong evidence for the existence of intermediary compounds such as HX and shows that their life may even exceed a few hours. This points to a rather high degree of stability comparable with that of reduced coenzyme I during fermentation. At the same time it may be concluded that such a compound has a pronounced specificity as otherwise it could not remain active in the presence of the various cell constituents of the suspension, a number of which might certainly be easily hydrogenated.

It would seem to be of great interest to make a more detailed study of the nature of this compound.

In a number of cases a characteristic induction period has been observed when a suspension was illuminated for the first time. It is possible that such an induction period is caused by the presence of an easily oxidizable substance; when this substance has been destroyed by oxidation it is understandable that an induction period is not to

*) In order to distinguish radicals and atoms from ions the symbols indicating the former will be underlined.

be observed at the second illumination. On the other hand it is tempting to ascribe this phenomenon to the absence of the $\underline{H}X$ compound from the original suspension. It takes some time for this compound to be produced in sufficient concentration to hydrogenate oxygen. Once the compound has been formed it persists for some time after the suspension has been placed in the dark and hence the absence of a second induction period when the suspension is placed in the dark following a period of illumination. A second induction period might be expected to occur if the $\underline{H}X$ compound could be removed before the suspension is placed in the dark. This assumption was confirmed by the following experiment.

Experimental Material used. 500 mg from fresh leaf of healthy oat plant, grown in the greenhouse under conditions of artificial illumination between October to December, in 20 ml 0.005 N. sodium bicarbonate buffer solution. Temperature 20°C. Platinum electrode. pH 7.13. At the commencement of the experiment pure oxygen was bubbled through the suspension to replace the air above the suspension (fig. 12).

The general course of the changes in redox potential was the same as in previous experiments although in this case an initial induction period was not observed. There was a rapid increase in potential of more than 200 mV which was reversed to a decrease as soon as the oxygen stored in the suspension was exhausted since the rate at which oxygen was consumed exceeded the rate of diffusion into the suspension. The addition of manganese whilst the suspension was in the dark caused a sudden increase in the potential as in previous experiments. In this case however when the maximum potential is reached, there is no sharp peak in the curve but a tendency for the potential to remain constant. This indicates that reduction and oxidation balance each other in contrast to the situation at A where reduction predominates. Since the rate at which oxygen diffuses into the suspension will be the same in both cases it must be the reducing capacity of the suspension which has diminished presumably as a result of consumption of the $\underline{H}X$ compound, formed during the first illumination period, by manganese induced hydrogenation in the subsequent period in the dark.

When the suspension was now illuminated for the second time the potential did not increase immediately as in previous experiments, instead there was an initial decrease of 70 mV followed immediately

by a rapid increase. Here a pronounced induction period was observed at the second illumination. After the illumination was turned off, to terminate the second illumination period, the potential continued to increase for a few minutes indicating that hydrogenation of oxygen

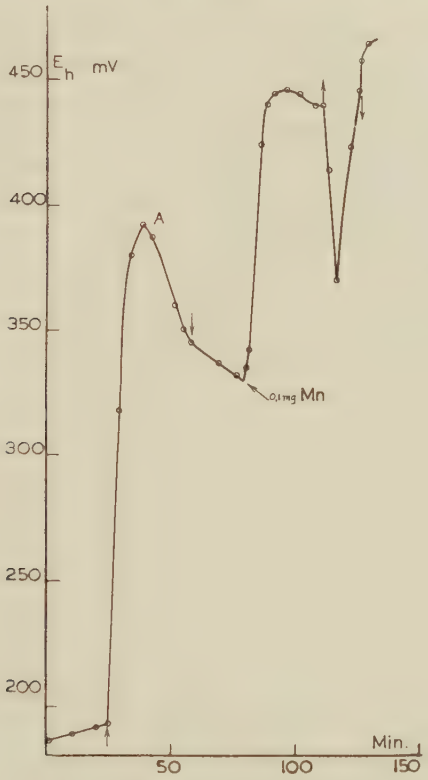

Fig. 12. Curve of potential changes in a chloroplast suspension showing a pronounced induction period after the second illumination period. (See text)

was continuing as there was now a reserve of the HX compound. A possible explanation as to why the first induction period was not observed in this case might be the difference in potential levels at the beginning of the two illumination periods since at the beginning of the experiment the potential was 250 mV more negative than that at the beginning of the second illumination period and it is to be expected that reduction begins more readily when the potential is already at a low level.

The redox potential changes in these crude chloroplast suspensions depend on a number of different factors of which the following may be mentioned:

1. The photo-oxidation and reduction activity of the suspension.
2. The extent of the back reaction $\underline{H} + \underline{OH} \rightarrow H_2O$.
3. The potentials and concentrations of the reduced and oxidised states of the heavy metals and their compounds.
4. The redox potentials and concentrations of the various reaction products, peroxides, hydrogen peroxide, reduced coenzyme etc.
5. The rate of diffusion of oxygen into the suspension.
6. The initial E_h and pH of the suspensions and pH changes resulting from respiration, addition of reagents etc.
7. The presence of acceptors for $\underline{H}$, $\underline{OH}$, and $\underline{O}$.
8. Temperature.
9. Light intensity.

This list demonstrates the complexity of the system involved and although accurate physico-chemical calculations are excluded it is possible to draw general conclusions in the case of a number of these factors by changing one factor at a time.

It is now clear from the above experimental evidence that the high potentials are only developed, in the suspensions from crushed *Avena* leaves, in the presence of oxygen, manganese and the direct or indirect action of light. Although it seems probable that these high potentials are caused by a peroxide it is not yet certain whether they are due to hydrogen peroxide, a metal peroxide or an organic peroxide.

The fact that high potentials are only developed, in the case of an illuminated suspension saturated with oxygen, when manganese is added, seems to indicate that the activated hydrogen complex $\underline{H}X$ is unable to hydrogenate oxygen directly. The presence of manganese seems to be essential for the hydrogenation of oxygen to take place. With reference to the specific part played by manganese in oxygen transfer *) it would appear very likely that manganese could function as an activator of oxygen thereby participating in the first step in peroxide formation. This would require the existence of an oxidation-reduction system in which divalent, trivalent and perhaps quadrivalent manganese is involved.

*) e.g. in the case of the Cu containing enzyme laccase [3]), Manganese as activator of the dioxymaleinic acid oxidase [27]), Manganese added to metmyoglobin causes an oxidative decarboxylation of oxalacetic acid to malonic acid and carbon dioxide [28]).

Although trivalent manganese compounds are unstable and show a strong tendency to give rise to di- and quadrivalent manganese spontaneously (i.e. $2\,Mn^{\cdot\cdot\cdot} \rightarrow Mn^{\cdot\cdot} + Mn^{\cdot\cdot\cdot\cdot}$) this tendency might be checked to some extent by adsorption on a carrier, by complex formation or by resonance stabilisation. Rabinowitch [19]) p. 294) has put forward a scheme for the mechanism of oxidase action in which iron takes part as a heavy metal; the essential part of this scheme might well be used in connection with the role of manganese in the present problem. Even though the manganese is attached to a carrier or combined to form a complex, it would still be possible for it to form a chemical association with oxygen. In the above mentioned scheme it is supposed that the intermediates of peroxide nature are stabilised by resonance between the ferrous and ferric ion forms. In the present case a similar system may be postulated between the di- and trivalent manganese according to the following scheme:

$$Mn^{++} + O_2 \rightarrow Mn^{++} \begin{matrix} /O \\ | \\ \backslash | \\ O \end{matrix} \quad \text{resonance} \quad Mn^{+++} \begin{matrix} /O \\ | \\ | \\ O- \end{matrix} \quad \text{resonance}$$

$$Mn^{+++}\,O- + (e + H^+) \rightarrow Mn^{++} \begin{matrix} OH \\ | \\ \backslash \\ O \end{matrix} + (e + H^+) \rightarrow Mn^{++} + HO{-}OH$$

Net result $O_2 + 2\underline{H} \rightarrow H_2O_2$.

Since the suspension is instantaneously activated after the addition of manganese in the present experiments this indicates that rather than the formation of a complicated complex we are dealing with a comparatively simple adsorption phenomenon in which the manganese is only loosely bound *). In this connection it is interesting to note that Arens [1]) showed that when the leaves of Elodea were placed in a solution containing $Mn^{\cdot\cdot}$ and illuminated, manganese dioxide was precipitated on the leaves. This makes it highly probable that manganese may also occur in a higher state of valency inside the leaves.

*) This is different to the case of the activation of l-leucine amino exopeptidase by Mn^{++} where complete activation may require many hours and a comparatively stable compound is formed [23]). On the other hand an oxidation reduction system is not involved in this case.

Biological oxidation of divalent manganese to compounds of higher valency has also been observed in the case of certain aerobic soil bacteria whereas in plants, the existence of an oxidation reduction cycle, in which manganese is involved has recently been made plausible by Kenten and Mann [12a]) who should that in horse radish extract oxidation of Mn^{++} to Mn^{+++} or Mn^{++++} takes place in the precence of H_2O_2.

The high potentials recorded in the crude *Avena* chloroplast suspensions, during illumination after the addition of manganese, may be due to the presence of small amounts of trivalent manganese as well as to the formation of hydrogen peroxide. However the former is highly unstable and since the potential of a chloroplast suspension to which hydrogen peroxide had been added was the same as that of an illuminated chloroplast suspension containing manganese it seems reasonable to assume that the high potentials observed are due principally to the formation of hydrogen peroxide. Moreover these high potentials are of exactly the same level as those obtained in catalase free cultures of hydrogen peroxide producing *Pneumococci*.

The rapid decrease in potential during continued illumination would under such circumstances be explained by exhaustion of the oxygen stored in the suspension; the formation of hydrogen peroxide ceases and the hydrogen peroxide already formed acts as a hydrogen acceptor and is reduced to water. From the slope of the curves (see fig. 4) the rate of formation of hydrogen peroxide, when the suspension is illuminated, is very high and is the same as the velocity of reduction of the hydrogen peroxide when the oxygen in the suspension is exhausted. This in understandable as in both cases two hydrogen atoms are used. The high rate of formation of hydrogen peroxide also means that in spite of the presence of catalase in the chloroplast suspension it is possible to detect hydrogen peroxide production.

The rate of the decrease of the potential when the suspension is in the dark is one tenth of the rate when the suspension is illuminated (fig. 4). The deminished rate of hydrogen peroxide conversion in the dark is not alone the result of the catalatic destruction of the hydrogen peroxide still present in the suspension, but is also due to the reduction of the hydrogen peroxide by the remaining $\underline{H}X$ compound which persists for some time after illumination has ceased.

The fact that the curves of potential changes show sharply in-

creasing and decreasing potentials would appear to indicate that two reactions are involved although in fact only one process is involved, the hydrogenation of oxygen to form water with hydrogen peroxide as an intermediary. One of the factors determining the level to which the potential of the suspension will increase is the quantity of oxygen dissolved in the suspension and diffusing into it.

It may be questioned as to whether the observations recorded when manganese is added to crude chloroplast suspensions bear any relation to photosynthesis and if so to what extent manganese participates in normal photosynthetic processes.

There is no reason to suppose that the ability to form peroxides will not be present in the intact plant. In one exceptional case the high potential characteristic of peroxide formation was observed in a suspension of normal leaf material in the absence of added manganese. On the other hand it was shown that, in a suspension of leaf material from the etiolated leaves of a plant highly deficient in manganese, potential changes under the influence of strong illumination were negligible until a small amount of manganese was added when normal activity was observed. Both these observations in conjunction with the previously recorded observation that carbon dioxide assimilation is reduced in manganese deficient plants make it highly probable that manganese plays a prominent part in photosynthesis.

As the potential of a chloroplast suspension from a normal leaf lies between the above mentioned extremes it seems reasonable to suppose that the quantity of manganese present in the leaf is one of the principal factors determining the redox potential in the leaf during illumination. This implies the presence of a photosensitive oxidation reduction system in which manganese plays an important part and in which there is equilibrium between manganous ions and manganese ions of higher valency (tri- or quadrivalent); this equilibrium moving in the direction of oxidation during illumination.

As the normal potential of a manganous-manganic system in neutral solution is sufficiently high ($+$ 1.5 V) to oxidise water, even in the dark, it would seem better to attribute the role of catalyst in the primary process to manganese rather than to iron.

The first step might be a light induced electron transfer e.g. from manganese to iron, followed by photo-oxidation of water by trivalent manganese.

$$Mn^{2+} + Fe^{3+} \xrightarrow{h\nu} Mn^{3+} + Fe^{2+}$$

$$Mn^{3+}(H_2O) \xrightarrow{h\nu} Mn^{2+}(H_2O)^{+}\ ^{20})$$

$$Mn^{2+}(H_2O)^{+} + OH^{-} \rightarrow Mn^{2+} + H_2O + \underline{OH}$$

$$2\underline{OH} \rightarrow H_2O_2 \quad \text{or} \quad 2\underline{OH} \rightarrow \tfrac{1}{2}O_2 + H_2O.$$

It is possible that manganese might also function as an acceptor for hydroxyl radicals resulting in the formation of intermediates of a peroxide nature in which the trivalent manganese compounds are temporarily stabilized by resonance although hydrogen peroxide is eventually produced and ultimately decomposed by catalase with liberation of oxygen.

The limited scope of the experiments does not permit any decisive conclusions concerning details of the participation of manganese in photosynthesis, such as those suggested in the above speculations, to be reached.

The same holds true for iron as for manganese although there are a few points of difference which may be mentioned. It has been shown that, at the redox potentials, which are generally observed with suspensions of *Avena* leaf material, trivalent iron and divalent manganese may exist in close proximity without appreciably affecting the potential whilst the suspension is in the dark. Therefore it seems feasible that, in the plant, in the absence of light iron and manganese occur near each other as tri- and divalent ions respectively. During illumination manganese seems to participate chiefly in photo-oxidation processes whereas iron seems to be particularly active in photoreductive processes.

Weiss [37]) first suggested that, in the carbon assimilation processes of green plants, reduction of carbon dioxide takes place with the aid of Fe^{++} ions. The fact that addition of small amounts of iron, in the form of ferric ammonium sulphate, to an illuminated chloroplast suspension increases the rate of decrease of the potential four times (fig. 11) demonstrates that iron is easily integrated into the photoreduction system. This would appear to indicate that iron is concerned in the first, comparatively stable reduction product $\underline{HX}$; according to Rabinowitch [19]) p. 239), this seems very probable. However, according to the same author, since complex iron compounds which are thermodynamically capable of reducing carbon dioxide or the carboxyl group in a neutral medium are not known it is necessary to extend the hypothesis to include the forma-

tion of a second hydrogen donor $\underline{H}_2X$ of greater reducing power and lower redox potential.

It seems highly questionable, however, whether a redox potential of such a low level, as would be necessary for the reduction of free carbon dioxide, could ever be established in such an aerobic system as the living plant; it therefore seems more logical to look for a set of intermediary reactions which might avoid these extremely low potentials.

Ruben [21]) first suggested that the carboxyl group in the complex (CO_2) might be phosphorylated thereby facilitating its reduction; the necessary high-energy phospates being synthesized by the aid of light energy or by utilization of part of the organic products of photosynthesis in oxidative metabolism as postulated by Rabinowitch [19]), p. 229).

Although the attempts of Emerson c.s. [4]) to prove the existence of energy rich organic phosphorus compounds in *Chorella* were not convincing, the experiments of Wassink [31]) with Chromateum, though not decisive either, make it quite feasible that this organism is capable of building up energy rich phosphate bonds, during illumination, which are broken down to some extent when carbon dioxide becomes available.

The great importance of the existence of these intermediate, energy rich, compounds is that they would facilitate the reduction of carbon dioxide as they would easily come within the potential range of the primary reduction product $\underline{H}X$ thereby rendering the postulation of the rather improbable $\underline{H}_2X$ compound, of extremely low redox potential, unnecessary.

The supposition that iron, forming part of an organic complex, combines with $\underline{H}$ and functions in the primary photo-chemical reduction product which temporarily stabilizes the H atoms, in conjunction with the supposition that manganese, as such or adsorbed on a carrier, combines with $O\underline{H}$ and functions in the primary photochemical oxidation product, makes the interdependency of manganese and iron for optimal photosynthetic activity, as observed by different authors, understandable.

In conclusion the following comments may be made on the fundamental difference between crude chloroplast suspensions and intact leaves.

Firstly there is lack of structure and organization in the suspen-

sions compared with the intact leaves where the main parts of the photosynthetic process such as the splitting of water into H and OH, the prevention of the back reaction by removal of the OH and the reduction of the carbon dioxide complex can only occur in differently located sites of chemical reaction.

Rabinowitch [19]) p. 152, makes the following comments on this subject: "The secret of how back reactions are prevented must be sought in the heterogeneous structure of the photosynthetic apparatus".

Wassink [32]) first assigned a definite role to structure in the mode of energy transfer in diatom photosynthesis.

Although a suspension still contains intact chloroplasts and a certain discontinuity of the medium is still present, organized structure is out of the question. Cell structure is a decisive factor in regulating the rates of diffusion of gases to the reaction centres of the cell. The importance of the rate of diffusion of gases is clearly demonstrated by the shape of the curves of potential changes in various cases (figs. 4 and 6). When the oxygen in these suspensions is becoming exhausted there is not a gradual slowing down of the rate of increase of potential but instead a sudden reversal in the potential change which indicates that photoreduction is proceeding so vigorously that the oxygen molecules which still enter the suspension by diffusion cannot influence the potential. This makes it understandable how, although a cell is surrounded by oxygen, the reduction of the carbon dioxide complex can proceed, for the diffusion of oxygen is hindered by the cell walls and the protoplast etc. whilst those oxygen molecules which enter the cell and reach the centres of photosynthetic activity are quickly reduced to water and thereby prevented from disturbing the process of carbon dioxide reduction.

In the case of a chloroplast suspension exposed to the air, however, oxygen, which is much more easily reduced than carbon dioxyde, will monopolize all the available reducing power and this is certainly one of the reasons why photosynthesis can never take place *in vitro* in suspensions to which oxygen has free access.

The inhibition of photosynthesis in the presence of excess of oxygen, as observed by Warburg [29]), Wassink c.s. [33]) and Mc Alister and Meyers [16]) might be ascribed to the same cause, the oxygen competing with carbon dioxide for the available reducing

power; the distribution of sites of chemical reaction within the cell being unable to regulate so many oxygen molecules.

A further difference between the crude chloroplast suspension and the intact cell is that in the former, during illumination, oxygen is consumed (as will be shown in a subsequent paper) whereas in the latter oxygen is produced. In the suspension the cell constituents are altered in their relationship to the sites of chemical reaction becoming exposed to the strongly oxidizing activity of the OH compounds and some of them undergo rapid oxidation even to carbon dioxide. In this way even though the processes of photosynthesis take place the usual end result is not achieved, and the normal evolution of oxygen does not occur as it is diverted to the oxidation of organic matter.

Summary

In order to gain an insight into the nature of the oxidation processes concerned in photosynthesis and the role of manganese in this connection the changes in redox potential of suspensions from crushed, filtered and centrifuged *Avena* leaves were observed during illumination and whilst in the dark.

In suspensions to which oxygen has free access, rapid potential changes, amounting to 100 mV and more in a few minutes, can be observed during illumination.

In a number of cases a short initial induction period was observed which generally did not occur on illumination for a second time.

On addition of small amounts of manganous sulphate potential changes did not occur whilst the suspension was kept in the dark but the potential increased to 500 mV and even higher during illumination. This makes the formation of peroxides and in particular hydrogen peroxide, highly probable.

The course of the potential changes can be readily understood on the assumption that during illumination the crude chloroplast suspension is still able to split water into its components, H atoms and OH radicals, and that manganese plays a specific role in the hydrogenation of oxygen.

The fact that chloroplast suspensions, which have been illuminated, retain their ability to produce peroxide, for up to one hour after being placed in the dark, on the addition of manganese or reintroduction of oxygen makes it highly probable that the hydrogen atoms are incorporated in a complex of rather high stability which is able to retain its reducing capacity over a considerable period and has a greater specificity.

Addition of ferric ions to the suspension under the same conditions as in the case of manganese does not give rise to the same high potentials but appears to promote the reductive mechanism of the light induced reactions.

It is thought that the added iron is eventually incorporated into an organic complex which combines with hydrogen atoms and functions as the

primary photochemical reaction product in which the hydrogen atoms are temporarily stabilized.

Manganese, after a photo-induced electron transfer, is thought to be that active agent in the induction of photo-oxidation of water which is generally considered to be the first step in photosynthesis.

At the same time manganese, as such or adsorbed on a carrier, might also easily combine with the OH radicals the result being the primary photochemical oxidation product. This latter product finally gives rise to hydrogen peroxide which is readily decomposed by catalase into water and free oxygen.

The hypothesis that manganese and iron are complementary oxidation reduction catalysts seems to be supported by the present investigation and the frequently observed interdependency of the iron and manganese content of the plant for optimal growth becomes understandable.

The experiments show that photosynthesis is unlikely to occur *in vitro* in the presence of oxygen, as oxygen, being so much more easily reduced than carbon dioxide, monopolizes all the available reducing power.

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REFERENCES

1) Arens, K., Physiol. polarisierte Massenaustausch und Photosynthese bei submersen Wasserpflanzen. Planta **20**, 721–658 (1933).
2) Aronoff, S., Photochemical reduction of Chloroplast Grana. Plant Physiol. **21**, 393–409 (1946).
3) Bertrand, G., L'importance physiolog. du manganèse et d'autres elements. Ergeb. d. Vitamin und Hormonforschung **2**, 192–212 (1939).
4) Emerson, R. L., Stauffer, J. R. and Umbreit, W. W., Relationship between phosphorylation and Photosynthesis. Am. J. Botany **31**, 107–120 (1944).
5) Ewart, A. J., On assimilation inhibition. J. Proc. Linnean Soc. **XXXI**, 364–461 (1896).
6) Farkas, A. and L., On the photochemical primary process of ions in aquous solution. Trans. Faraday Soc. **34**, 1113 (1938).
7) French, C. S., Holt, A. S., Powell, R. D. and Anson, M. L., The evolution of oxygen from illuminated suspensions of frozen dried and homogenized chloroplasts. Science **103**, 505–506 (1946).
8) Gaffron, H., The effect of specific poisons upon the photoreduction with hydrogen in green algae. J. Gen. Physiol. **26**, 195 (1942).
9) Gaffron, H., Reduction of carbon dioxide coupled with the oxyhydrogen reaction in *Algae*. J. Gen. Physiol. **26**, 241 (1942).
10) Gerretsen, F. C., Manganese in relation to photosynthesis. Plant and Soil **1**, 346–358 (1949).
11) Hewitt, L. F., Oxidation reduction potentials in Bacteriology and Biochemistry. London. 4th Ed. 1937.
12) Hill, R., Oxygen produced by isolated chloroplasts. Proc. Roy. Soc. (London), B. **127**, 192–210 (1939).
12*a*) Kenten, R. H. and Mann, P. J. G. The oxidation of manganen by Plant Extracts in the presence of hydrogen peroxide. Biochem. J. **45**. 255–263. 1949.

13) Kostychev, S., Studien über Photosynthese. Ber. deut. botan. Ges. **39**, 319 (1921).
14) Kumm, J., and French, C. G., The evolution of oxygen from suspensions of chloroplasts. Am. J. Botany **32**, 291–295 (1945).
15) Mc Allister, The chlorophyll carbon dioxide ratio during photosynthesis. J. Gen. Physiol. **22**, 613 (1939).
16) Mc Allister and Myers, J., The time course of photosynthesis and fluorescence observed simultaneously. Smithsonian Inst. Pub. Misc. Collections. **99**. Nr. 6.
17) Morris, H. D., and Pierre, W. H., The effect of calcium, phosphorus and iron on the tolerance of *Lespedesa* to Manganese toxicity in culture plants. Soil Sci. Soc. of Am. **12**, 382–386 (1948).
18) van Niel, C. B., On the morphology and physiology of the purple and green sulfur bacteria. Arch. Mikrobiol. **3**, 1 (1931).
19) Rabinowitch, E. I., Photosynthesis and related Processes. New York 1945.
20) Rabinowitch, E. I., Electron transfer Spectra. Rev. Modern Phys. **14**, 112–131 (1942).
21) Ruben, S., Photosynthesis and Phosphorylation. J. Am. Chem. Soc. **65**, 279–282 (1943).
22) Shive, J. W., Significant roles of trace elements in the nutrition of plants. Plant Physiol. **16**, 435–445 (1941).
23) Smith, E. L., Manganese and l. leucine-Amidopeptidase. J. Biol. Chem. **163**, 15–27 (1946).
24) Somers, I. J. and Shive, J. W., The iron-manganese relation in plant metabolism. Plant Physiol. **17**, 582–602 (1942).
25) Stiles, W., Trace elements in plants. Cambridge, 1946.
26) Twyman, E. S., Iron-manganese balance and plant growth. New Phytologist. **45**, 18–24 (1946).
27) Theorell, H. C. S., Mangan als Aktivator der Dioxymaleine-säure Oxydase. Naturwissenschaften **27**, 95–96 (1936).
28) Vennesland, C. S., The action of metmyoglobin, oxygen and manganese on oxalacetic acid. J. Biol. Chem. **163**, 573 (1946).
29) Warburg, O., Über die Geschwindigkeit der photochemischen Kohlensäure-Zersetzung in lebenden Zellen. Biochem. Z. **103**, 188–217 (1920).
30) Warburg, O. and Lüttgens, W., Experimente zur Assimilation der Kohlensäure. Naturwissenschaften **32**, 161–301 (1944).
31) Wassink, E. C., Phosphate exchanges in purple sulfur bacteria in connection with photosynthesis. Kon. Nederl. Ak. v. Wetensch. Proc. **LII**, 412–422 (1949).
32) Wassink, E. C., Some remarks on chromphylline as the path of energy transfer in photosynthesis. Enzymologie **XII**, 363–372 (1948).
33) Wassink, E. C., Vermeulen, D., Reman, G. H. and Katz, E., On the relation between fluorescence and assimilation in photosynthesizing cells. Enzymologia. **5**, 100–110 (1938).
34) Waters, W. A., Chemistry of free radicals. Oxford, 1946, p. 83.
35) Weiss, J. J., Chemical and Biological Action of Radiation. Trans. Faraday Soc. **43**, 314–324 (1947).
36) Weiss, J. J., Radiochemistry of aquous solutions. Nature **153**, 748–750 (1844).
37) Weiss, J. J., Some remarks on the photosynthesis of green plants. J. Gen. Physiol. **20**, 501–509 (1937).

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COPPER CONTENTS AND COPPER DEFICIENCY IN DANISH SOIL TYPES

by F. STEENBJERG and ELSE BOKEN

The Royal Veterinary and Agricultural College, Copenhagen, Denmark.

During the past 16 years investigations have been made in Denmark on the occurrence of copper in the soil and in cultivated plants. Among other things, the investigations have included the influence of various factors on the solubility (availability in the soil) of the copper and the influence of the same factors on the total and relative copper contents of the crops.

It is beyond dispute that a knowledge of the distribution of copper in nature under various conditions is of fundamental importance to the study of its biological effects. Hence investigations have comprised various problems concerning the distribution of copper in the soil and in cultivated plants with special reference to fertilization with copper. Numerous observations have demonstrated that certain conditions may increase or decrease copper deficiency (yellow tip disease). Among such conditions the p_H-value of the soil, its degree of moisture, the nature of the previous crop and the addition of sand or clay may be mentioned. It has also been proved [1]) that grass in particular is capable of extracting copper from the subsoil and of transferring part of this copper in available form to the surface soil.

Copper deficiency is found all over the world and causes considerable losses in crop production as well as to live-stock breeders. In Denmark copper deficiency is mainly found in Jutland, especially in sandy soils rich in humus, and in peaty soils. It is, however, also widely found in sandy soil with normal humus contents of from 2 —4 per cent in the Jutland area. This latter area (fig. 1) showing copper deficiency is only part of a larger northwestern European region where large or small areas of soil with considerable humus contents are interspersed among podsolic sand-soil areas.

The present work is not concerned with analyses of the total copper contents of soil, fertilizers and crops under Danish condi-

Fig. 1. Geological Soil Map of Jutland
(From the Geological Investigation of Denmark)

tions. Interested students are referred to previous works, [1]) and [2]), where the copper-value which is used in the present work for determining the available copper contents of the soil was empirically derived.

Investigations of copper contents and copper deficiency in Danish soil types have made use of the copper-value, T_{Cu} *), and copper-values have been determined in 2245 samples of surface soil and 595 samples of the sub-soil and at the same time p_H-values, phosphorus values †), and potassium-values †) have been determined parallel with the copper-values for 430 samples of the surface soil. In addition, various determinations were made in connexion with the results of 520 field trials with copper sulphate and various copper minerals. These field experiments were carried out from 1925 to 1944 by the Danish farmers' unions.

Alongside the work of evaluating copper deficiency by means of the copper-value, pot experiments [2]) for evaluating the fertilizing value of the different copper minerals were carried out in 1937–45. Later, field experiments were made for the purpose of determining the fertilizing value of these copper minerals, several of which proved to be well suited for this purpose when finely ground, and are considerably cheaper per kilo of copper than copper sulphate which is relatively expensive.

A. *Determination of the Copper Contents of the Soil.*

1. Extent and Method of the Investigation. Soil samples from the Jutland peninsula, 2245 soil samples from the surface soil (depth 20 cm) and 595 soil samples from the subsoil (depth 30–40 cm) were investigated. The copper analyses and the other chemical soil analyses were carried out during the period of 1941–44. The soil samples were taken partly systematically from a few large areas, and partly at random from the whole of Jutland, and the soil-sampling sites were accurately marked on a map, scale 1 : 20.000. Statistical examination proved that the results of the analyses of the soil samples, taken systematically as well as at random, might be treated together without reservation [3]).

*) See page 209.

†) Phosphorus-value and potassium-value are used in Denmark as measures of the available phosphorus and potassium in the soil.

For determining the copper-value, two portions of air-dried soil (passing a 2 mm mesh) each of 50 grammes, were extracted with 200 ml hydrochloric acid of such a concentration that the final acidity of the filtrate approximated to a p_H-value of 2. Formerly, the copper in the soil extract was precipitated with hydrogen sulphide [1]), the isolation of the copper in the present was made by electrolysis before the colorimetric determination. The soil extract after evaporation with aqua regia, concentrated nitric acid, and distilled water was made 1 *N* with sulphuric acid and then poured into an electrolytic vessel of a special design [4]). The electrolytic process was carried on for 1 hour with a terminal voltage of a little more than two volts and a current of a little more than 10 milliamperes. By gas or electric heating the temperature of the liquid in the electrolytic vessel was kept around the boiling point for the first 30 minutes of the electrolysis. For the last 30 minutes the heat was turned off. The copper, precipitated on a cathode of fine-mesh platinum wire netting, was dissolved in 4 *N* nitric acid and, after the addition of an excess of concentrated ammonia water, the copper was colorimetrically determined [1]).

The reproducibility of the method is illustrated by the duplicate determinations shown below (table I) of soil samples taken in field trials with copper fertilizers; a is the treatment with basal

TABLE I

Duplicate Determinations of Copper-values (1944)

Locality	Treatment	1st Det.	2nd Det.
Sndr. Duborg II . . .	a	3.1	3.0
	b	37.0	34.5
	c	7.1	6.7
Faarhus	a	2.8	2.0
	b	4.4	3.9
Vokslev	a	4.6	4.0
	b	8.6	7.4
	c	2.7	2.8
Sndr. Duborg I . . .	a	3.8	3.6
	b	12.0	11.7
	c	6.2	6.4
Holstebro	a	4.0	5.2
	b	4.7	4.7
	c	3.6	4.6
Fandrup	a	1.7	1.0
	b	9.6	10.4
	c	4.4	5.6
Vust	a	3.1	2.3
	b	6.0	5.4
	c	3.2	4.6

dressing, b the treatment supplied with 50 kilogrammes of copper sulphate per ha, and c the treatment supplied with 102 kg of copper pyrites per ha. (Cu in both b and c corresponds to the copper contents of 50 kg copper sulphate).

It may be mentioned that, in 1946, a new chemical method was worked out for determining copper-values, this method is quicker and just as accurate, but has not been used for the analyses given in the present article.

2. Copper-value and Increase in Yield. As already mentioned, the applicability of the copper-value as an indicator of copper deficiency of the soil has so far only been founded on empirical observations, an account of which has been previously given [1]).

A theoretically unimpeachable method for a chemical determination of copper deficiency is not available at the moment. The difficulties of determining the available copper in the soil are very great, partly on account of the extremely small quantities of copper contained in the soil, but more particularly because there is a strong tendency for the cupric ions to form slightly soluble complex compounds. It may be that there are many copper compounds in the soil with so large a complexity constant that the cupric ions set free cannot be absorbed directly by plant roots until some decomposition (mineralisation) of them has taken place. Possibly too, there are in the soil soluble compounds of a humus-like nature containing copper fixed in a complex manner, the molecules of such compounds may be small enough to be absorbed directly by plant roots [1]). Finally, there is a certain probability that part of the copper present in slightly soluble minerals is of importance to the nutrition of the plants [2]). These hypothetical considerations make it plain that the determination of available copper presents extraordinary difficulties both in definition and analysis.

In further support of the applicability of the copper-value as an indicator of copper deficiency a summary is given below of various investigations into the correlation between the copper-value and the yield increases from trials with copper fertilizers.

In investigating the connection between the size of the yield increase in field trials using copper fertilizers, and the size of the copper-value in soil samples from the unmanured treatments it

must be borne in mind how extremely difficult it is to obtain a really good experimental basis, so that the different treatments have the same productivity before the trial begins. By inspection of field trials it is often found that the experimental basis is poor, so poor that it must be taken for granted that the trial will give but an incomplete and often wrong picture of the copper deficiency. Anybody who has had the opportunity of seeing copper deficiency in the field, as for instance by observing deficiency symptoms and growth in a barley field, will know the scattered and irregular distribution of the copper deficiency similar to that of phosphorous acid and potassium; but, in case of copper, there are still more pronounced differences in growth within very small areas so that, by moving, for instance, 10–20 cm, one may pass from evidently sound to totally destroyed barley. These irregularities in the growth are found to be so pronounced in case of copper deficiency, particularly because the curve of yield for copper is extremely steep. In other words, extremely small changes in the available copper content of the soil in the steeply rising part of the yield curve produce very great differences of yield. The latter fact is also worth keeping in mind in all cases where field trials are made to determine correlations between copper-value and increase in yield; the inaccuracy in the determination of the copper-value (see table I) will, in the case of small copper-values (great copper deficiency), give particularly great difficulties in determining the relation between copper-value and yield increase.

Hence for various reasons, the determination of the relation between the size of the yield increase and the size of the copper-value is subject to considerable uncertainty. In addition, the accuracy in field sampling is often too low, and, as already mentioned, the determination of small copper-values has not, so far, been as exact as might be desired in view of the particularly great importance of making an accurate determination of the small copper-values on account of the steep yield curve. The last-mentioned problem has, however, been taken up in recent years and an improvement has been obtained so that future determinations of the copper-values *may* be made with greater exactness than has been the case up to the present.

The whole question of interaction between copper on the one hand and nitrogen, potassium, and phosphorus, manganese etc. on the other hand is, furthermore, a very important source of discrepancies,

between the copper-values and the results of the field trials. The varying yearly precipitation seems to be of some importance, too (see below).

Finally, experiments and observations, such as those of Willis and Piland [5]) in the U.S.A., have shown that, under certain conditions, a correlation may occur between the application of copper sulphate, the manganese absorption and the occurrence of manganese chlorosis. Quite independent of the investigations in U.S.A., Oedelien [6]) has in Norway arrived at the very interesting result that copper sulphate may produce or increase manganese deficiency (and iron deficiency) especially in soil of a high p_H-value. Similar observations have been reported also from Germany and Holland. Possibly the explanation is that the cupric ion acts as a catalyst in the oxidation of manganese and iron in the soil. If, on closer investigation, such conditions are found to be of general occurrence, this will create further difficulties for the evaluation of the results of field trials.

Inspite of these sources of discrepancy between the copper-value and the size of the yield increase, the fact remains that, when soil samples are taken for copper determination, some from soil of very high copper deficiency, and some from soil that is not deficient in copper, the copper-values are considerably higher in the latter case.

In the first reports on the copper contents of the soil [1]) it was, indeed, possible to find a considerable difference in the copper-values of the soil at these two extremes of the scale as will be seen from the table given below.

	I Severe Copper deficiency	II Copper deficiency	III Sound crop
Average Copper-values . .	1.6	3.1	14.8
Number of Soil Samples .	42	25	22

The evaluation of the degree of the copper deficiency of the crops was made by one person, so that in group I were included only those results from soil samples where copper deficiency symptoms of the crops were so severe that it was predictable with a great degree of certainty that the grain crop would be practically nil, while the group III of soil samples, where the crops were sound, was almost exclusi-

vely represented by clay soils. There is not only a pronounced tendency in the average figures, but the difference between the totally ruined crop and the perfectly normal crop is also large.

The terms used to indicate the degree of copper deficiency are qualitative terms. Nevertheless, for copper as for other plant nutrients, it may be desirable to determine a quantitative average relation between the size of the copper-values and the yield increase. Besides the general difficulties connected with an exact determination of the said relation as mentioned above, there is the special difficulty that, as yet, only few results are available from field experiments with copper fertilizers where soil samples were taken for the determination of the copper-value. So, in order to have a fairly large number of estimates within the individual groups, only four groups were formed. The average yield increase was determined at copper-values from 0–2.5, 2.6–5.0, 5.1–10.0, and, finally, above 10. From the above table of qualitative relationship it was known that at copper-values above 5 one will often find soils less deficient in copper than at lower copper-values. A number of field experiments designed to compare equal applications of different copper compounds to a range of soils [12.5 kg Cu as copper pyrites and copper sulphate], started in 1943 in Jutland, give the same impression; the first-year results (yield increase values) are given in the table below, the crops being in all cases cereals.

Yield Increase Values (kg per ha)			
Copper-values	Copper Sulphate	Copper Pyrites	Number of Trials
0 –2.5	364	215	5
2.6–5.0	127	97	9
above 5.0	145	90	4

In table II the experimental results from all trials where 50 kg copper sulphate per ha was used as fertilizer, have been divided into 4 groups according to the size of the copper-value in soil samples from the unmanured plots. Here there is a problem due to the various crops used, although they were mostly cereals (barley and oats), and, it has therefore been necessary to convert the yield increase values into fodder units *).

*) Here as well as in later tables 1 kg grain, 5 kg straw, 2.5 kg hay, 1.1 kg dry matter of root, 10 kg grass = 1 fodder unit.

TABLE II

Copper-values	Yield in Fodder Units	Average Yield Increase in Fodder Units 1st Year	Number of Trials
0 – 2.5	3307	250	13
2.6– 5.0	2844	220	30
5.1–10	2224	179	24
above 10	2547	64	4

The average yield increase in the group with copper-values of 0–2.5 is rather low. Besides the possible explanations of this already given, we may mention the possibility that the added copper in some cases becomes so firmly fixed in soils with low copper-values that it is difficult for the plants to absorb the necessary copper, so that the average yield increase for this group is lower than expected from its low copper-value. That this may be the case has been ascertained by one of the authors (Steenbjerg), who had the opportunity to make observations under controllable conditions. In a sandy soil rich in humus (sandy peat moor) a dressing of 25 and 50 kg copper sulphate had no effect on the development of copper deficiency symptoms. Only after a dressing of 200 kg copper sulphate per ha did the crop in question — barley — acquire a normal appearance. Hence it must be recommended that in future trials with copper fertilizers, the application of two very different amounts of copper sulphate (e.g. 50 and 250 kg per ha) should be made.

3. The Geological Origin of the Soil and the Copper Content. The extensive sandy-soil areas in Jutland (fig. 1) mainly consist of two geologically distinct types, diluvial sand and melt-water sand. Both types have been deposited in the glacial periods by glacial water from inland ice.

It was of interest to compare the copper contents of these two types of sandy soils †). There proved to be no difference, the average copper-values of soil samples which had had no copper fertilizer after 1935 were 3.77 for diluvial sand (511 soil samples) and 3.74 for melt-water sand (376 soil samples). Soils which had received copper-fertilizer after 1935, gave average copper-values for diluvial sand of

†) For the division of these soil types, Phil. D. Keld Milthers, State Geologist, kindly placed at our disposal his original working maps, scale, 1 : 160000.

6.33 and for melt-water sand of 7.91, i.e. a considerable increase of the copper-value through copper applications. The distribution curves of the copper-values for the two groups (with or without dressing of copper after 1935) were also plainly displaced in relation to each other.

The number of moors and sandy peat moors was so small — 10 and 11, respectively — that no comparison could be made between the average copper-values for copper-fertilized and non-copper-fertilized soils of this kind, but the average copper-values were especially low, being 2.97 for moors and 3.81 for sandy peat moors.

4. The Soil Type (the Humus Content of the Soil) and the Copper-value. After having been air-dried the soil samples taken in 1943 and 1944 were roughly divided into 6 groups, namely, gravel, clay, sandy soil, "grey sand", "black sand", and moor, i.e. a division mainly in accordance with the humus contents (colour) of the soil. In support of the subjective classification a collection of these soil types was made, and their humus contents were determined [8]). The classification of the soil samples according to types on this basis was always made by the same person, and the soil samples were air-dried before the classification took place.

The humus determinations of these soil samples gave the following results.

TABLE III

Soil Type	Humus Percentage
Gravel	0.4
Clay	2.2
Sandy Soil	4.1
"Grey Sand"	5.4
"Black Sand"	19.6
Moor and Sandy Peat Moor .	38.6

As will be seen, there is a considerable difference in the humus contents of the soil types enumerated. To clarify further this classification it may be explained that the most prominent quality of black sand in a dry state is due to part of the humus content which appears as well-defined humus particles under slight magnification, i.e. they lie plainly separated from the whitish or greyish quartz particles.

When seen with the naked eye the soil seems to be black on account of the large proportion of humus. Grey sand is a similar type of soil, but due to the considerably lower humus content, the colour is dominated by that of the quartz particles. On the whole the soil is of a greyish shade.

By sandy soil is meant a soil with fine grains of sand, the quartz content being dominant, but the quartz particles (when slightly magnified) are not separated from the humus substances as is the case in black sand and grey sand. Furthermore, sandy soil contains, as a rule, small and varying quantities of clay.

TABLE IV

	Moor and Sandy Peat Moor	Black Sand	Grey Sand	Sandy Soil Rich in Humus	Clay
	No Copper Fertilizing after 1935				
Average Copper-value . .	2.75	2.64	3.39	4.17	4.03 *)
Number of Soil Samples . .	86	123	93	473	65
	With Copper Fertilizer				
Average Copper-value . . .	3.40	4.02	5.11	6.29	7.20 *)
Number of Soil Samples . .	17	19	16	109	1

*) Mainly samples from the northwest of Jutland.

From table IV it will be seen that the average copper-value decreases (also proved by displaced distribution curves) with increasing humus contents in the soil, which seems to indicate that the humus substances in the soil influence the availability of the copper to the plants. No attempts have been made to determine the influence, if any, of the quality of the humus substances on the availability of the copper determined by means of the copper-value. The special influence of the humus substances on the availability of the copper in the soil is, however, confirmed by the present investigations (further ref. S v a n b e r g [9]). The special importance of the humus substances in fixing the copper in the soil also appears from the fact that, when the same quantity of copper is added to each of these soil types, a decreasing rise in the copper-value will be found in the following order sandy soil — grey sand — black sand — moor and sandy peat moor (see pp. 209).

Apart from moor and sandy peat moor, of which no large areas are found in Denmark, the soil types particularly deficient in copper according to the copper-value are, therefore, black sand and grey

sand, which is in accordance with previous observations of the appearance of copper deficiency in Jutland [10]).

As there is no difference between the copper-values of diluvial sand and melt-water sand, it was supposed that the relative number of black sand, grey sand, and sandy soils was the same in diluvial sand and melt-water sand. This really proved to be the case as shown by the figures as follows.

	Black Sand	Grey Sand	Sandy Soil
Diluvial Sand	14.2 per cent	14.0 per cent	71.8 per cent
Melt-water Sand	18.8 per cent	12.5 per cent	68.7 per cent

5. The Copper-value in the Subsoil. An examination was made of the size of the copper-value in surface soil and subsoil samples taken at the same place. The samples were taken at depths of 1–20 cm and 30–40 cm. The results are given in table V.

TABLE V

	Diluvial Sand		Melt-water Sand		Clay		Peaty Soil	
	Surf. Soil	Sub-soil	Surf. Soil	Sub-soil	Surf. Soil	Sub-soil	Surf. Soil	Sub-soil
No Copper Fertilizing after 1935								
T_{Cu} *)	3.3	3.3	3.4	3.3	4.5	5.3	2.6	2.7
Number of Soil Samp.,	178	178	154	154	150	150	6	6
Fertilized with Copper after 1935								
T_{Cu}	5.3	4.5	5.1	3.2	11.3	7.4	1.2	1.2
Number of Soil Samp. .	19	19	31	31	1	1	1	1

*) See page 209.

Where no copper fertilizing had taken place after 1935, the average copper-values of surface soil and subsoil samples taken at the same place were practically the same. In case of copper dressing the copper-values of the surface soil increased.

When the individual results are considered, it appears that, within all geological soil types, there are cases where the copper-value is considerably higher in the subsoil than in the surface soil. Therefore, it is probable that it is not always sufficient to determine the copper-value of the surface soil. The greater copper contents of the subsoil may presumably, in certain cases be the reason why no increase of yield is obtained by dressing with copper even if the copper-value of

the surface soil is low, and this may also be the explanation in some of the cases when, in the beginning of the period of growth, copper deficiency symptoms are evident, and then disappear later when the roots of the plants reach the subsoil.

6. Cultivation of Heaths etc. Much of the sandy soil cultivated in Jutland has been cultivated in the course of the past 70 years. As the heath cultivation is still going on, it was of interest to examine the copper contents in raw heath soil mainly covered with heather (*Calluna vulgaris*). 40 soil samples were taken from heaths at different places in Jutland. The samples included the layers of bleached sand (a_1 and a_2) and part of the humus pan (b_1). Generally, the copper-values were very low, so that the average copper-value for diluvial sand was 1.77 and for melt-water sand 1.79. Possible correlations between the degree of podsolisation expressed by the thickness of the a_1 and a_2 layers and the copper-value were investigated. No such correlation could be found, neither did the p_H-value, the phosphorus-value, nor the potassium-value of the heath soil depend on the degree of podsolisation.

From a comparison of the average copper-values in raw heath soils and the average copper-values in cultivated sandy soils (without copper dressings after 1935) — for diluvial sand 3.77 and for melt-water sand 3.74 - - it will be seen that, roughly speaking, the copper-values have been doubled in the cultivated soil. This increase is partly due to copper dressings before 1935, partly to the extraction by the crops of copper from deeper layers of the soil [1]). It is possible in addition that the copper-fixing capacity of the humus substances has been changed in a way favourable to the plants by the cultivation of the soil (liming, dressing with phosphorous acid, and tilth).

The great copper deficiency that appears in newly cultivated heath soil after a variable number of years of cultivation must, in the main, presumably be explained by the original low copper contents of these soils which, from a plant physiological point of view, may be further diminished when the soil is dressed with the other elements such as nitrogen, phosphorus, and potassium. This is confirmed by an examination of those sites where no copper dressings have been applied after 1935 and for which information of the time of cultivation of the soil is available.

TABLE V*a*

The Copper Value and the Time of Cultivation				
Time of Cultivation	1860–79	1880–99	1900–19	1920–34
Average Copper-values	4.0	4.4	4.1	4.5
Number of Soil Samples	14	47	96	71

The copper-value is rather constant irrespective of the time of cultivation. The decrease of yield due to copper deficiency that is often observed up to 5–20 years after the cultivation, may, consequently be ascribed not to a decline of the copper-value (the average copper-value in raw heath soil was 1.8), but, probably, to the reason that after cultivation larger dressings of other nutrients than copper have been given whereby copper is gradually and relatively reduced to a minimum. The average phosphorus-values and potassium-values in the table below indicate that this is probably the case.

TABLE VI

Phosphorus-values and Potassium-values and Time of Cultivation					
Time of Cultivation	Raw Heath Soil	1860–79	1880–99	1900–19	1920–34
Average Phosphorus-values .	0.8	3.7	4.1	3.6	2.3
Number of Soil Samples . .	25	26	100	92	159
Average Potassium-values .	3.1	4.2	5.4	5.4	5.2
Number of Soil Samples . .	24	26	101	91	158

The average phosphorus-value in raw heath soil is 0.8. After 20–50 years of cultivation this figure is 3–5 times as high, while the copper-value is only a little more than twice as high. The potassium-values, too, are higher after the cultivation of the heath than they were before, but the increase would have been still higher if all the potassium-values had been determined in spring after the dressing. This is because the crops remove a great part of the potassium present in the soil during the period of growth and thereby bring about a great reduction of the potassium-value (the exchangeable potassium). Furthermore, there is a greatly increased application of nitrogen in artificial fertilizers, farmyard manure, and liquid manure after the cultivation.

The copper-value is an approximate figure indicating the absolute degree of the copper deficiency. The absolute amount of any nutrient in the soil is of great importance in the supply to the plants of the

nutrient in question and to the yield; but the relative supply of nutrients (and the precipitation) is also important. One gets an idea of the relative copper supply by a simultaneous determination of the other nutrients in the soil. The copper-values of diluvial sand and melt-water sand have proved to be alike; but if one of these geological soil types contained a greater quantity of the other nutrients than the other, copper might be reduced to a relative minimum and so by application of copper it would be possible even here to obtain an increase of yield.

Investigations into the supply of the other nutrients in these two geological soil types have proved that there is hardly any difference in the average contents of phosphorus, potassium, and nitrogen, nor is there any difference in the average p_H-values. Also the distribution curves of the above mentioned analytical values for melt-water sand and diluvial sand more or less coincide. Thus, according to these investigations, the distribution and degree of the copper deficiency may be expected to be alike for melt-water sand and diluvial sand.

7. Copper-value and Copper Application. Copper supply to the surface soil may be due partly to the cultivated plants extracting copper from the subsoil and transferring it to the surface soil (root extraction, e.g., grass [1]) and partly by dressing (or spraying) with copper-containing substances.

A copper-value, T_{Cu}, of 1 means that one milliequivalent, or 31.785 mg Cu is found in 625 kg air-dried soil. In case of an application of 50 kg copper sulphate per ha, corresponding to 12.7 kg Cu, the copper-value should, theoretically, be increased by 125 units, if no copper were fixed in the soil. [One ha (of a depth of 20 cm) may be considered equal to 2 million kg soil.]

It appears from table IV that the increase of the average copper-values following such applications to peaty moor, black sand, grey sand, and sandy soil are 0.65 — 1.38 — 1.72, and 2.12 units, respectively. Hence the increase of the copper-value caused by copper application declines with rising humus contents. Compared with the theoretical possibility of an increase of 125 units, the variation from 0.65 to 2.12 units is very small, but mutually compared the variation range of the increase – from 0.65 to 2.12 — is considerable.

The increase of the copper-value brought about by the same cop-

per dressing does not vary with the soil type alone but also with the period between the copper dressing and the soil sampling. If soil samples are taken only one year after the copper application, the increase of the copper-value is considerably greater than it appears from table IV. It must also be remembered that these soils which were not dressed with copper were not soils that had never had copper dressings, but soils which had certainly received no copper dressings after 1935. The quantity of copper applied to the soils that were dressed with copper after 1935 was, as a rule, about 6.3–12.7 kg Cu per ha added as copper sulphate.

In 1944, copper-values were determined in soil samples from 24 field trials made in Jutland in 1943 which had been dressed with 12.7 kg Cu per ha as copper sulphate and copper pyrites respectively. Out of these 24 trials 20 were on sandy soil, 1 on black sand, 2 on grey sand, and 1 on peaty moor. In the sandy soil (20 trials) the average increase of the copper-value was 8.2 units for copper sulphate and 2 units for copper pyrites; as to the other 4 trials the average increase of the copper-value was 2.8 and 2.4 units, respectively. The ascertained increases of the copper-value showed the greatest variation after the application of copper sulphate.

Previous investigations [1]) of the copper content of soil bearing permanent crops (grass) had given such interesting results that it was felt this investigation ought to comprise another common permanent crop in these areas, namely coniferous trees. 41 soil samples were therefore taken below the mor layer in the heath plantations (spruce and mountain pine), the majority of which were more than 25 years old. The average copper-value was found to be 6.47 in diluvial sand (25 samples) and 6.12 in melt-water sand (16 samples). These values are nearly twice as high as the corresponding values in cultivated arable land (diluvial sand 3.77, melt-water sand 3.74), and as the plantations had not been fertilized with copper, this increase may well be due to needle-shedding (copper extraction from the subsoil [1])).

B. *Field Trials with Copper Dressings in Jutland 1925–44.* 1. The Geological Origin of the Soil and the Increase of Yield. During the period of 1925–44 640 field trials with copper sulphate were made by the local agricultural advisers in Jutland. In 517 trials, the location and the nature of the soil

were successfully determined according to the geological soil map of Bornebusch and Milthers[7]). This map was used in order to secure uniform determinations of the soil types. The average figures of yield increases are given in table VII.

TABLE VII

Types of Soil and the Average Increase of Yield			
Soil Types (Bornebusch and Milthers)	Number of Trials		Average Yield Increase 50 kg Copper Sulphate per ha Fodder Units per ha
	Total number	Cereals	
Diluvial Sand	288	267	392
Melt-water Sand	99	80	399
"Clay" (see table IX)	91	82	408
Peat Moor, Sandy Peat Moor, and Meadow	14	14	379
Post-glacial and Late-glacial formations *)	20	20	449
Dune and Drift Sand.	5	5	145

*) Mainly Sandy Soil.

It will be seen that for 50 kg copper sulphate per ha the increase of yield was the same for diluvial sand and melt-water sand, as was to be expected from the previous investigations of the copper-values etc. of these geological soil types. The distribution curves of the yield increases in diluvial sand and melt-water sand also approximately coincide. Both copper-value and increase of yield point in the same direction. There is no discernible difference in copper deficiency in these two very large groups of sandy soils.

The 91 trials in clay soil gave an average increase of yield corresponding to the yield increase for diluvial sand and melt-water sand. This increase of yield is surprisingly high. The reason for this is partly that the geological soil map indicates the soil type at a depth of 50–100 cm below the surface. As far as the sandy soils, sandy peat moors, and peat moors are concerned, this will, however, give no discrepancy in a determination of the soil type from the surface soil. But in the north-western part of Jutland there are some districts with sandy surface soil and clayey subsoil.

If a closer survey is made of the 91 trials that were located on clay soil according to the geological soil map, it will be seen that the soil types in these trials carried out by the advisers have been described as follows (tabel VIII):

TABLE VIII

Estimate of Soil Samples		
Clay Soil	9 Trials	
Sandy Soil Mixed with Clay	1 ,,	
Clay Soil Mixed with Chalk	2 ,,	22 Trials
Humus Soil	10 ,,	
Sandy Soil	65 ,,	
Peaty Soil	4 ,,	

The 22 trials in brackets gave an average increase of yield of 125 fodder units with 50 kg copper sulphate. However, it should be stated that the 9 trials in the table made on clay soil, as estimated by the advisers, gave an average increase of yield of only 69 fodder units per ha.

2. 1st Year Increase of Yield by Application of Various Copper Fertilizers. a. *Copper Sulphate*. Of the 640 trials with copper sulphate made in Jutland in 1925–44, 84 trials, or 13 per cent, gave negative results. Table IX

TABLE IX

Year	Average Yield Increases in Fodder Units Exclusive of Negative Results	Number of Trials	Average Yield Increases in Fodder Units Inclusive of Negative Results	Number Trials	Negative Results in per cent
1925	1224	1	1224	1	0
1926	738	12	671	13	8
1927	628	19	555	21	10
1928	727	42	692	44	5
1929	498	43	467	45	4
1930	541	66	455	76	13
1931	631	66	554	73	10
1932	592	46	566	48	4
1933	364	42	303	48	13
1934	353	27	217	36	25
1935	423	26	362	30	13
1936	411	17	342	20	15
1937	385	32	346	35	9
1938	428	25	339	30	17
1939	322	20	191	30	33
1940	314	12	205	17	29
1941	184	26	156	29	10
1942	228	13	204	14	7
1943	253	17	135	24	29
1944	129	4	38	6	33
Total Av.	480	556	400	640	13

contains a survey of the average increase of yield in fodder units during these years, both when all trial results are included, and when the negative results are excluded.

The greater part of the trials were made with cereals. From the 2nd column in the table it will be seen that the average annual increase of yield has decreased from 1925 till 1944. There may be several reasons for this.

In the course of the years copper-containing sprays have been increasingly used in agriculture. Furthermore, especially in the first years, the trials may have been made on areas of particularly great copper deficiency. Thus, during the first 5 years, only 5.6 per cent of the trials gave negative results while 14.9 per cent of the trials gave negative results during the last 15 years.

However the reason for the fall in the increase of yield during this period may be the variation in the precipitation. Of the 556 results given in table IX a total of 503 trials with cereals were used for an examination of this problem. Information was given by The Meteorological Institute of Denmark [11]) about the precipitation in Jutland during the period.

The results were first divided so that those from years with more than normal precipitation for a certain period of the year, or the whole year, belong to one group, while trials made in years with less than normal precipitation during the same periods belong to another group. The periods chosen are given in table X.

The average increase of yield with 50 kg copper sulphate per ha has been calculated for each of the two groups and for each period;

TABLE X

Increase of Yield and Amount of Precipitation in Various Periods

Precipitation for	Normal Precipitation for Jutland mm	More than Normal Precipitation		Less than Normal Precipitation		Difference in Yield Increase Fodder Units
		Average Increase of Yield in Fodder Units	Number of Trials	Average Increase of Yield in Fodder Units	Number of Trials	
The Whole Year .	680	504	252	475	251	29
The Whole Period of Growth . . .	178	552	202	448	301	104
May	44	565	238	423	265	142
June	48	499	303	477	200	22
May + June . . .	92	542	344	377	159	165

it will be seen that the precipitation is of importance to the size of the increase of yield. Within the period of growth the precipitation in May seems to be of the greatest importance.

There is only an apparent difference between these results and the experience [12]) that copper deficiency is most pronounced in dry years when this investigation shows, on the contrary, that the increase of yield by application of copper sulphate is smallest. Presumably, the explanation is that the smaller crops to be seen on copper-deficient soils in dry years are due to deficiency of water as well as of copper, but presumably there has been a tendency to emphasize the symptoms of copper deficiency observed, and the copper deficiency has been considered the main reason for the small crop.

TABLE XI

Average Yield and Increase of Yield in Periods of Five Years

Years	Average Yield Fodder Units. Basal Dressing	Average Increase of Yield in Fodder Units. 50 kg Copper Sulphate per ha	Number of Trials	Average Precipitation in May-June
1925–29	2540	645	109	112.3
1930–34	2601	537	221	96.4
1935–39	3049	391	110	88.5
1940–44	2600	231	63	77.1

From table XI it will now be seen that both the average increase of yield and the average precipitation in May and June decline evenly from the period 1925–29 to the period 1940–44. This means that a considerable part of the decrease of the yield increases that took place over the whole period *may* be due to decrease of the precipitation during the periods of growth. Quite similar results concerning the effect of precipitation on the increase of yield were found by pot experiments made in 1939–44 [3]).

Hence, it cannot be determined with certainty whether copper deficiency in the soil (i.e. the proportion of the amount of available copper and the amount of the other nutrients in the soil) as estimated in field trials has been significantly less pronounced during the past 20 years.

For further illustration of the kind of increase of the yield obtained by application of different quantities of copper sulphate, the results of 74 field trials with cereal crops may be given. In each of these

trials applications of both 50 and 100 kg of copper sulphate per ha were made. A list of the average results from the trials is to be found in table XII.

TABLE XII

Number of Trials	Average Yield in Fodder Units/ha	Average Yield Increase in Fodder U./ha with 50 kg Copper Sulphate	100 kg Copper Sulphate
74	2519	481	631

b. *Ashes of Sulphur Pyrites.* Until 1940, copper sulphate was the only copper fertilizer used in Denmark. Already in 1937 pot experimenst had been made in an attempt to find other copper fertilizers. These pot experiments were continued until 1945 [1]) [2]) and [3]). As there was a lack of copper sulphate in 1940, ashes of sulphur pyrites were tried in field trials for 3 years on account of the experiences gained by previous pot experiments. Ashes of sulphur pyrites are residual products from the production of sulphuric acid made by roasting sulphur pyrites. Presumably, the copper is mainly present in the form of cupric oxide (abt. 2/3) and copper sulphate (abt. 1/3). The sulphur pyrites in question contained 1.6–1.8 per cent Cu. The amount of copper applied in the two dressings corresponded to an application of 50 kg copper sulphate per ha. The results of the 26 trials made are to be found in table XIII.

TABLE XIII

Copper Sulphate and Ashes of Sulphur Pyrites				
	Copper Sulphate		Ashes of Sulphur Pyrites	
Year	Av. Increase of Yield Fodder Units	Number of Trials	Av. Increase of Yield Fodder Units	Number of Trials
1940	425	6	481	6
1941	220	18	221	18
1942	310	1	64	1
1940–42	273	25	277	25

Ashes of sulphur pyrites will never be of any importance as a copper fertilizer, the costs of transportation and delivery are too high. The interesting point in these first trials, is however, that cupric oxide seems to be an excellent copper fertilizer if finely ground, a possibility already revealed by pot experiments [1]). However, one cannot ignore as another possibility that the ashes of sulphur pyrites may contain other micro-elements of importance under the given

conditions of trial. Furthermore, the copper in the ashes of sulphur pyrites is probably more easily distributed in the field than copper as copper sulphate, since it is a question of spreading 700–750 kg copper pyrites ashes per ha against 50 kg of copper sulphate.

c. *Copper Pyrites.* According to the results of the pot experiments [1]), [2]), and [3]), copper pyrites is the mineral that is least suitable as a copper fertilizer. It was used in field trials, because it is the most common copper mineral and it is mined in the largest quantities. Furthermore, on the basis of the results from the pot experiments, the results from the field trials with this copper mineral make it possible to foretell how much better the effects of the other copper minerals will be when applied in the field. The copper pyrites applied contained 12.8 per cent Cu and about 30 per cent zinc sulphide. The effect was compared with the effect of copper sulphate in 26 trials, when the same amounts of copper were given in the two fertilizers. The results are to be found in table XIV.

TABLE XIV

Copper Sulphate and Copper Pyrites				
Year	Copper Sulphate		Copper Pyrites	
	Av. Increase of Yield. Fodder Units	Number of Trials	Av. Increase of Yield. Fodder Units	Number of Trials
1941	352	5	329	5
1942	298	8	258	8
1943	268	13	210	13
1941-43	293	26	248	26

Hence, finely ground copper pyrites may be used as a fertilizer even if the effects are somewhat inferior to those of copper sulphate. When considering these results, one must bear in mind firstly that the copper pyrites contained about 30 per cent zinc sulphide, which seemed to have hardly any poisonous effect, and secondly that copper pyrites is very cheap compared with copper sulphate, so that, copper pyrites holds a more favourable economic position than that expressed by the trial results themselves. Moreover, an equally good effect might have been obtained with copper pyrites as with copper sulphate, if the copper pyrites had been still more finely ground. The copper pyrites used in these field trials had a degree of fineness that corresponded fairly well to the most finely ground copper pyrites used in the pot experiments [2]). The price of grinding compared with

the price of copper in the copper pyrites will be decisive as to how finely it may be ground.

Since copper pyrites containing zinc sulphide has proved so effective in these field trials, it is of great importance to test other copper minerals which, according to the results of the pot experiments [2]) and [3]), have appeared even more suitable.

3. Residual Effects of Copper Fertilizers. Since copper dressings are comparatively expensive and since various investigations show that, among the micro-elements of interest under Danish conditions, copper gives the greatest residual effects, a determination of the residual effects of this element applied in different fertilizers is of particular importance to illustrate the economic aspect of copper dressing.

a. *Copper Sulphate.* The 1st year residual effect was determined by 40 trials where, at the beginning of the trials, 50 kg copper sulphate was applied per ha. The results are given in table XV in fodder units.

TABLE XV

1st Year Effect	1st Year Residual Effect	Total effect for 2 Years	Number of Trials
315	260	575	40

The residual effect one year after dressing with 50 kg copper sulphate is considerable and, according to the results of these trials, on an average of the same magnitude as the 1st year effect.

Only 18 trials have been made where it has been possible to compare the residual effects after the 1st and 2nd years with the 1st year effect. Three trials have been continued for 4 years and here it is possible to gain some estimate of the 3rd year residual effect. The average figures are given in table XVI, *a* and *b* in fodder units.

TABLE XVI, *a* and *b*

	1st Year Effect	1st Year Residual Effect	2nd Year Residual Effect	3rd Year Residual Effect	Total effect	Number of Trials
a 1925–44	226	275	217	—	718	18
b 1942–45	41	88	193	112	434	3

The trials show that copper sulphate has a considerable residual effect, but the comparatively small number of field trials made

cannot allow any decisive conclusion to be drawn as to its magnitude and durability. The question is of such great interest that it should be more closely examined.

b. *Copper Pyrites.* For the determination of the 1st year residual effect of copper pyrites 17 trials are available, and from 11 of these the 2nd year residual effect may also be determined. The average results are given in table XVII, *a* and *b* in fodder units.

TABLE XVII, *a* and *b*

	1st Year Effect	1st Year Residual Effect	2nd Year Residual Effect	Total Effect	Number of Trials
a 1941–44	221	240	—	461	17
b 1942–45	235	310	171	716	11

A comparison between the yield increases in tables XVI, *a* and *b*, and XVII, *a* and *b*, shows that the residual effects of copper pyrites and copper sulphate are of the same magnitude.

C. *Copper Dressing in Future.* As already mentioned, copper sulphate was, until 1940, the copper fertilizer exclusively used. It is, however, comparatively expensive to use in soils where the copper fertilizing effect is below the average.

Copper minerals can be obtained in a fairly pure state by a flotation process. By the flotation of ore a so-called copper concentrate is obtained, which only needs to be finely ground to be suitable as a fertilizer. The various copper minerals contain very considerable quantities of copper. In a pure state several of them contain more copper than copper sulphate, as will be seen from table XVIII.

TABLE XVIII

Copper Sulphate ($CuSO_4.5H_2O$)	25.0 per cent copper
Cupric Oxide (CuO)	79.9 ,, ,, ,,
Cuprite (Cu_2O)	88.8 ,, ,, ,,
Copper Pyrites ($CuFeS_2$)	34.6 ,, ,, ,,
Copper Glance (Cu_2S)	79.9 ,, ,, ,,
Bornite (Cu_5FeS_4)	63.3 ,, ,, ,,
Malachite ($CuCO_3.Cu(OH)_2$)	57.5 ,, ,, ,,

All the minerals listed here contain more copper than copper sulphate, most of them 2–3 times as much. This means that even com-

paratively impure copper concentrates may contain considerably more copper than copper sulphate.

Malachite is on a level with copper sulphate from a fertilizing point of view. Malachite fields are found at various places in the world. The application of finely ground malachite would mean a great reduction in costs and at the same time the application of copper fertilizer would be profitable on much larger areas than before. *Copper Glance* and *Bornite* and *Cuprite* might be also used after a suitable grinding.

Copper Pyrites is the mineral of most general occurrence. Its effect is slightly inferior to that of copper sulphate, but further grinding, which is usually a relatively cheap process, would undoubtedly increase its effect to the level of that of copper sulphate.

Cupric Oxide gives excellent effects both in pot experiments and field experiments. In the pot experiments it was used in a pure state, in the field experiments as ashes of sulphur pyrites (and ashes of copper pyrites) obtained by roasting sulphur pyrites (and copper pyrites).

By roasting of copper pyrites varying quantities of copper sulphate are formed, depending on the temperature, the significance of which has, however, not been closely examined. Experiments with these slightly soluble fertilizers were started on the idea that by giving to soil slightly soluble copper fertilizers it might in certain cases be possible to delay the fixation of the copper in the fertilizer by the copper-fixing substances in the soil and hence the plants might absorb as much as possible of their extremely small but essential amounts of copper direct from the copper fertilizers and rely as little as possible on the relatively insoluble compounds formed by the cupric ions and the soil.

However, the application of a fertilizer depends not only on its effect but also on its price. It is evident that a refined industrial product, such as copper sulphate, is considerably more expensive than the raw material — some copper mineral — from which it is produced. By the use of suitable copper minerals as fertilizers the price per kg copper will be very low, presumably $^1/_3$-$^1/_2$ of the price of the copper in copper sulphate.

A low price per kg copper in the new fertilizers means a greatly increased profit and also the possibility of widespread application of cheap copper fertilizers under conditions where the yield increases

are relatively small. Furthermore, in several cases, for instance in the cultivation of heaths it would be natural to consider copper application as a soil amendment. The low prices of these copper fertilizers and their residual effect coupled with the observed low copper content of the heath sand make such a point of view a natural one.

Summary and conclusions

1. Experience has shown that certain factors influence the degree of copper deficiency (yellow tip disease). These factors are p_H value, soil type (humus content), the nature of the previous crop, addition of clay and sand etc. The present investigations, using a special method of determining available copper, have further demonstrated that the above factors affect the available copper in the soil.

2. It has been proved that there is a correlation between copper values and yield increases by copper dressings. Both values have been determined by means of field and pot experiments.

3. Analyses of soil, in geologically well defined areas in Jutland, which included the estimation of copper values and of pH-values, phosphorus-values and potassium-values, precipitation etc., showed a general distribution and intensity of copper deficiency such as would be expected from the above experiments. Field trials on these soils, involving copper dressings produced the expected yield increases in the main.

4. The results of pot experiments and field experiments with different copper minerals (copper pyrites, bornite, copper glance, cuprite, malachite) and copper chemicals (copper sulphate, cupric oxide, and metallic copper) show that several of these slightly soluble copper compounds are suitable as copper fertilizers if ground to a suitable fineness, and that their residual effect, like that of copper sulphate, is considerable.

5. These and other similar copper minerals do not ordinarily occur in soils. Since copper in the cuprous state may occur in the crystal lattice of the coarser particles of the soil, due regard must be paid thereto when chemical methods for the determination of copper deficiency of the soil are worked out.

6. Attention is called to the difficulty of defining available copper and also to certain difficulties of analytical procedure in determining copper values. The chemical nature of soil copper is unknown and the definition of available copper is based on empirical observations.

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REFERENCES

1) Steenbjerg, F., Kobber i Jord og Kulturplanter. Med særligt Henblik paa Gulspidssyge. Tids. Planteavl **45**, 259–368 (1940).
2) Steenbjerg, F., Kobber i Jord og Kulturplanter II. Undersøgelser. over Kobbermineralers Gødningsværdi. Tids. Planteavl **47**, 557–599 (1943).
3) Steenbjerg, F. and Boken, Else, Kobber i Jord og Kulturplanter III. Kobberindhold og Kobbermangel i jydske Jordtyper. Tids. Planteavl **52**, 375–459 (1949).
4) Grendel, F., Over het Kopergehalte van eenige Voedingsmiddelen. De Koperverontreiniging van eenige IJzerpraeparaten. Een Micro-Koperbepaling in Bloed. Pharm. Weekblad **67**, 913, 1050, 1345 (1930).
5) Willis, L. G. and Piland, J. R., The function of copper in soils and its relation to the availability of iron and manganese. J. Agr. Research **52**, 467 (1936).
6) Ødelien, M., Jernmangel på myrjord og kobbersulfatets virkning på planternes jern- og manganforsyning. Tids. f. Det norske Landbruk, 3–4 (1945).
7) Bornebusch, C. H. and Milthers, Keld, Jordbundskort over Danmark. Danmarks geologiske Undersøgelse. III Rk. Nr. 24.
8) Schollenberger, C. J., A rapid approximate method for determining soil organic matter. Soil Sci. **24**, 65–68 (1927).
9) Lundblad, K., Svanberg, O. and Ekman, P., The availability and fixation of copper in Swedish soils. Plant and Soil. **1**. 227–302 (1949).
10) Jørgensen, C. A., Gulspidssygen. Dens Udbredelse, Aarsager og Bekæmpelse. Tids. Planteavl. **34**, 76–116 (1928).
11) Hansen, H., Vejrforholdene i Landbrugsaarene 1925–44. Tids. f. Landøkonomi. 1925–44.
12) Gram, Ernst, Bormangel og nogle andre Mangelsygdomme. Tids. Planteavl. **41**, 401–449 (1936).

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DES EFFETS DE L'ÉPANDAGE DE NITRITES SUR DES CULTURES EN PLEIN CHAMP

par A. VAN DEN HENDE

Institut Supérieur Agronomique de l'État à Gand

Les idées sont encore partagées quant à la toxicité des nitrites pour les plantes, et quant à la possibilité pour l'azote des nitrites d'être utilisée par les plantes de culture. Nous avons exposé dans une communication précédente [1]) le résultat des travaux effectués au cours d'essais de laboratoire par plusieurs chercheurs sur l'un et l'autre de ces deux problèmes. La littérature ne mentionne par contre qu'un seul essai réalisé en plein champ [2]).

Nous avons dès lors estimé utile d'appliquer des nitrites sur des herbages ainsi qu'en plein champ, avant ou après l'ensemencement de cultures.

Nous espérons de cette façon fournir des renseignements sur:

1) la toxicité éventuelle des nitrites dans les conditions pratiques de culture, et sur la nature et l'importance des dégâts occasionnés par l'épandage de $NaNO_2$;

2) le danger afférent à la présence de petites quantités de nitrites dans les nitrates du commerce;

3) la possibilité de se servir de nitrites comme herbicide, si comme les données de la littérature le font supposer, les nitrites se révèlent plus toxiques pour certaines familles de plantes;

4) l'utilisation de l'azote des nitrites par la plante.

Au cours des années 1945 à 1948 des essais en plein champ ont donc été aménagés, sur plusieurs cultures notamment sur des navets, des betteraves fourragères et sucrières, du froment, de l'avoine, de l'orge, ainsi que sur des herbages.

En vue d'établir l'influence des conditions dans lesquelles les expériences ont été accomplies, nous avons effectué les essais sur plusieurs sols, notamment sur un sol argilo-sablonneux à M e l l e, ainsi que

sur des sols sablonneux situés à Melle et à Heusden, les conditions climatériques variant naturellement d'une année à l'autre. Nous avons au cours de plusieurs essais épandu le $NaNO_2$ à des périodes différentes c'est-à-dire avant ou après l'ensemencement. Enfin l'action du $NaNO_2$ à été jugée par comparaison avec l'état de parcelles traitées au $NaNO_3$ appliqué au même moment et partant dans les mêmes circonstances.

Les essais ont toujours été effectués avec deux répétitions, la grandeur de chaque parcelle variant entre 10 et 20 m^2 *).

Les essais sur les cultures ont été effectués sur plusieurs types de sol, les expériences aménagées sur des herbages uniquement sur les sols argilo-sablonneux approximativement neutres des Stations de recherche et de la ferme de l'état à Melle (Gand).

Le tableau I nous fournit un aperçu général des résultats obtenus sur les cultures en plein champ.

Les résultats enregistrés sur les herbages sont rassemblés dans les tableaux II.

Discussion des resultats. Nous discutons successivement l'effet toxique et l'utilisation au moins théorique de l'azote des nitrites par la plante.

1) L'effet toxique des nitrites. Des tableaux I et II il ressort suffisamment que dans bien des cas, l'épandage de nitrites à occassionné des dégâts aux cultures.

L'ampleur des dégâts différé considérablement. C'est ainsi que l'épandage du nitrite au moment de l'ensemencement semble avoir eu un effet néfaste sur les semis de navet traités par 800 (essai no. 1) et par 500 (essai no. 3) kg de $NaNO_2$ par ha, ainsi que sur le semis de froment (essai no. 9) traité par 200 kg par ha; sur ces parcelles le nombre de semences germées est en effet inférieur à celui observé sur les parcelles témoins ou traitées au nitrate. Ces constatations confirment celles effectuées par Stutzer [5]) au cours d'essais de laboratoire et d'après lesquelles la présence de nitrites est nuisible à la germination de plusieurs plantes; Stutzer mentionnait que les jeunes plantes de navets sont particulièrement sensibles aux nitrites.

Il est donc possible que le nitrite a endommagé les semences ou

*) Les circonstances experimentales ont été décrites en détail dans des communications précédentes [3]) [4]).

Aperçu des résultats expérimentaux obtenus apres épandage de $NaNO_2$ sur les cultures en plein champ

No de l'essai	Nature du sol	Année	Quantité de $NaNO_2$ appliquée par ha	Conditions climatériques pendant la croissance	Dégâts observés	Production comparée à celle des parcelles: témoins	Production comparée à celle des parcelles: traitées par $NaNO_2$	Rapport en poids feuillage / racines sur les parcelles
				Culture : Navet				
1	argilo-sablonneux (pH = 6,6)	1945	500 kg avant l'ensemencement	n* = 0,4 modérement chaud et humide	levée moindre	navets: 83% feuillage: 103%	84 % 69 %	témoins 1,04; $NaNO_2$ 1,22; $NaNO_3$ 1,46
2		1945	500 kg après l'ens.	n = 0	phénomènes de brûlures	navets: 46% feuillage: 58%	45 % 50 %	témoins 1,04; $NaNO_2$ 1,30; $NaNO_3$ 1,20
3		1947	500 kg avant l'ens.	n = 0 très chaud et sec	levée moindre	pas à établir avec précision		témoins 1,20; $NaNO_2$ 1,32
4	sablonneux (pH = 7,5)	1947	500 kg avant l'ens.	n = 0 très chaud et sec	dégâts minimes	navets: 102% feuillage: 132%	—	
5	sablonneux (pH = 5)	1948	200 kg avant l'ens.	n = 0 modérement chaud et humide	difficile à établir	navets: 166% feuillage: 131%	118 % 98,5%	témoins 0,87; $NaNO_2$ 0,95; $NaNO_3$ 1,04
			200 kg 15 jours avant l'ens.	n = 2,6		navets: 93% feuillage: 103%	121 % 110 %	témoins 0,78; $NaNO_2$ 0,61; $NaNO_3$ 0,74
				Culture: Orge				
6	argilo-sablonneux	1947	500 kg	n = 0 très chaud et sec	aucun	pas à établir avec précision		
				Culture: betteraves fourragères				
7	(pH = 6,6)	1947	100 kg 14 jours après l'ens.	n = 0 tres chaud et sec	phénomènes de brûlures	betteraves: 77% feuillage: 71%	—	témoins 0,18; $NaNO_2$ 0,16
				Culture : betteraves sucrières				
8		1947	500 kg 6 semaines après l'ens.	n = 0 modérement chaud et humide	dégâts minimes	betteraves: 105% feuillage: 123%	95,5% 94 %	témoins 0,675; $NaNO_2$ 0,790; $NaNO_3$ 0,805
				Culture : froment				
9		1947-	200 kg avant l'ens.	n = 0,6 modérement humide	levée moindre	paille: 101 % grain: 105%	85 % 88 %	
10		1948	200 kg 6 semaines après l'ens.	n = 0	aucun	paille: 111% grain: 121%	— —	
				Culture: Avoine				
11		1946	330 kg avant l'ens.	—	dégâts minimes	pas à établir avec précision		
12			330 kg après la levée	n = 0,4				

*) n = précipitation le jour de l'épandage

TABLEAU II

Aperçu des résultats expérimentaux obtenus après épandage de $NaNO_2$ sur des prairies

No. de essai	Année	Date d'application et quantité appliquée	Conditions climatériques pendant la croissance	Observations	Production comparée à celle des parcelles	
					témoins	traitées par $NaNO_3$
13	1945	13-8-1945 800 kg/ha	modérement chaud et humide	phénomènes de brûlure disparaissants après quelques jours	1e coupe: 140% (5-9-45)	56%
					2e coupe: 130% (5-10-45)	74%
					3e coupe: 89% (5-11-45)	99%
					total des coupes: 130%	62%
14	1946	26-4-46 405 kg/ha	idem	brûlures moins accentuées	coupe du (26-5-46	76%
15	1947	11-8-47 500 kg/ha et	très chaud et sec	brûlures très prononcées	1e coupe: 138% (2-10-47)	76%
					2e coupe: 139% (12-5-48)	96%
	1948	12-5-48 250 kg/ha	modérement chaud et humide	brûlures moins accentuées	1e coupe: 191% (22-7-48)	104%
					2e coupe: 105% (29-10-48)	100%

bien empêché la germination ou encore brulé les jeunes plantes au moment de la germination.

Par contre, l'essai no. 6 semble démontrer, que les semences d'orge sont résistantes à l'action éventuelle des nitrites puisque dans les conditions de l'expérience, aucun effet toxique n'a pu être observé.

Si l'on examine l'effet des nitrites épandus après la germination on constate à nouveau une graduation très marquée dans l'effet: des dégâts semblant provenir d'effets de brûlures ont apparu sur les navets (essai no. 2 à 800 kg/ha) et sur les betteraves fourragères (essai no. 7 à 1000 kg/ha); les dégâts furent très limités au cours de l'essai (no. 8) effectué sur les betteraves sucrières (à 500 kg/ha) et nuls sur le froment (essai no. 10 à 200 kg/ha).

Les observations effectuées après épandage de $NaNO_2$ sur une prairie au cours de l'été chaud et sec de 1947 illustrent particulièrement l'effet d'une quantité considérable de nitrite notamment 500 kg/ha (essai no. 15). Cinq jours après l'épandage, de sérieux phénomènes de brûlures furent en effet constatés principalement sur les quelques plantes de trèfle, sur les renoncules et sur les pissenlits; les

graminées aussi étaient endommagées par endroit. Notons cependant que les parcelles traitées au nitrate présentaient aussi de légers dégâts. Lorsque après 25 jours d'absence de pluie, les nitrites et nitrates furent dissouts, la végétation reprit rapidement ; les parcelles ayant reçu les nitrites et nitrates se caracterisèrent bientôt par une teinte plus foncée que les parcelles témoins; aucune végétation n'était encore à ce moment visible sur les endroits brûlés des parcelles traitées au $NaNO_2$. La répétition de l'épandage de $NaNO_2$ sur les mêmes parcelles au cours du printemps 1948 plus humide et moins chaud eut un effet défavorable moins accentué.

On remarquera à la lecture des tableaux I et II que l'action des nitrites diffère très considérablement dans les différents essais, et que cette action semble dépendre de beaucoup de facteurs dont la connaissance nous échappe encore. Il ne fait aucun doute que la nature de la plante joue un rôle important ; par contre il ne nous est pas apparu à quel moment de la germination ou de la croissance, les végétaux présentent la plus grande sensibilité : ainsi l'effet de l'épandage des nitrites avant l'ensemencement au moyen de froment fut bien plus défavorable que l'épandage sur les semis six semaines plus tard ; par contre les dégâts occassionnés par l'épandage des nitrites 20 jours après l'ensemencement furent plus notables qui ceux provoqués par l'épandage avant l'ensemencement.

L'influence de la nature du sol et même des conditions climatériques ne semble pas très prononcée. Nous n'avons pas pu confirmer les observations de Mevius et Dikussar[6]), d'après lesquelles la toxicité des nitrites augmente considérablement avec l'acidité du milieu ; l'épandage de nitrite sur les navets en terrain très acide (essai no. 5) n'a pas occassioné les dégâts attendus.

Les expériences démontrent que les nitrites peuvent provoquer des dégâts ; notons cependant que des dégâts importants n'ont jamais été occasionnés par des quantités de nitrite inférieurs à 500 kg/ha ; de cette remarque il ressortirait qu'il ne faut pas attacher d'importance à de petites quantités de nitrites présentes comme impuretés dans d'autres engrais.

Il est de plus difficile d'établir, partant de ces essais, jusqu'à quel point les nitrites ont donné lieu à un effet vraiment toxique ou si les nitrites ont simplement brûlé les plantes ou les germes comme le feraient d'ailleurs certains engrais (cyanamide par ex.) utilisés à un moment inopportun. L'action néfaste des nitrites semble en tout cas

de courte durée, probablement par suite de leur transformation plus au moins rapide en d'autres composés azotés: nous verrons d'ailleurs que plusieurs raisons portent à croire que l'azote des nitrites est après transformation éventuelle utilisée par les végétaux comme source d'azote.

2) L'utilisation de l'azote des nitrites par la plante. Au cours d'une communication antérieure [1]) nous avons suffisamment discuté les données fournies à ce sujet par des essais de laboratoire.

Ces données peuvent se résumer comme suit: les nitrites se comportent comme source d'azote pour beaucoup de plantes ayant passé la première phase de croissance; en milieu acide la toxicité devient toutefois trop forte. Nous venons d'ailleurs de signaler que l'action toxique des nitrites semble de courte durée, ce qui peut s'expliquer par la transformation très rapide des nitrites non seulement par voie bactérielle mais aussi suivant un processus physico-chimique comme plusieurs auteurs [7]) [8]) le prétendent. En tout cas l'azote des nitrites serait utilisable par les plantes soit par absorption directe (d'après Perciabosco et Rosso [9])), soit après transformation en d'autres combinaisons.

Les essais effectués en plein champ confirment la plupart de ces observations: malgré les dégâts occasionnés par l'épandage des nitrites, une augmentation de la production a été constatée dans plusieurs cas (voir tableau I), et surtout après application sur les prairies (voir tableau II). Il est incontestable, que les phénomènes de brûlures occasionnés par les nitrites mis à part, l'action des nitrites et des nitrates présente une grande simulitude; les augmentations de production en témoignent suffisamment.

Les résultats de l'essai no. 15 sont à ce point de vue particulièrement significatifs: rappelons que l'épandage avait été effectué en août 1947; au premier fauchage en octobre la production des parcelles traitées au $NaNO_2$ était comprise entre celle des parcelles témoins et des parcelles ayant reçu $NaNO_3$; la production des parcelles traitées au nitrite et au nitrate en Mai c'est-à-dire juste avant un nouvel épandage, moment ou l'effet toxique des nitrites ne se faisait plus sentir, fut du même ordre de grandeur. Deux et cinq mois après le deuxième épandage de 200 kg/ha ce qui à la faveur de conditions climatériques très favorables ne provoqua pas de brûlure, les dites parcelles produisirent à nouveau environ la même quantité.

Même en tenant compte du fait que $NaNO_2$ contient théoriquement 20,3% de N et $NaNO_3$ 16,8% on remarque qu'au cours de cet essai l'azote des nitrites et des nitrates a pu être utilisé dans une mesure à peu près pareille.

Ces observations correspondent d'ailleurs à celles de Schloesing [10]) qui mentionne qu'au cours d'essais en pots les nitrites et nitrates se sont comportés comme source d'azote de même valeur pour le maïs jaune.

Les parcelles traitées aux nitrites ont cependant dans presque tous les cas moins rapporté que les parcelles traitées aux nitrates évidemment en partie parce que les nitrites ont la plupart du temps provoqué des phénomènes de brûlures. Il n'est pas possible d'établir si cette raison est la seule, ou si l'azote des nitrites est (au point de vue théorique) moins bien utilisable que l'azote de nitrates parceque qu'il faudrait pour cela pouvoir déterminer l'importance parfois peu perceptible des brûlures.

Notons aussi que la similitude de l'action des nitrites et des nitrates comme source théorique d'azote apparait aussi après comparaison du rapport existant entre la production en feuillage et en racines; il est généralement admis que toute fumure azotée augmente ce rapport; les chiffres de la dernière colonne du tableau I démontrent que dans la plupart des cas l'épandage des nitrites a augmenté ce rapport comme l'aurait fait l'application d'un engrais azoté.

Conclusions. Ces essais effectués en plein champ prouvent que les nitrites produisent dans plusieurs cas des dégâts parfois conséquents aux cultures et que les plantes présentent une sensibilité variable à leur action. Il n'est cependant pas encore possible de déduire des résultats si l'utilisation des nitrites comme herbicides sélectifs peut être envisagée. L'action des nitrites semble cependant de courte durée probablement en raison de la prompte transformation des nitrites; plusieurs résultats portent d'ailleurs à croire que l'azote des nitrites à été eventuellement après transformation, utilisée par les plantes. Il n'en reste pas moins vrai, que l'emploi de nitrites comme engrais ferait courir le risque grave de voir occasionner d'importantes brûlures. Mais nous estimons que la présence de faibles quantité de nitrite, à l'état d'impureté dans d'autres engrais, ne peut pas provoquer de sérieux dégâts aux cultures.

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LITTÉRATURE

1) Van den Hende, A., Mededeel. Vl. Chem. Verenig. **11**, 12 (1949).
2) Eggleton, E. G., The Uptake of inorganic nitrogenous salts, including sodium nitrite by the grass plant. Trans. third Internation. Congr. Soil Sci. **1**, 203–204 (1935).
3) Van den Hende, A., Vraagstukken van Stikstof-huishouding. 2. De giftigheid der nitrieten voor onze cultuurgewassen. Mededeel. Landb. Hogeschool en de opzoekingsstations van de Staat te Gent **14**, 91–110 (1949).
4) Van den Hende, A. en Slaats, M., Vraagstukken van stikstof-huishouding. 3. De uitwerking van nitrieten op grasland. Mededeel. Landb. Hogeschool en de opzoekingsstations van de Staat te Gent **14**, 159–68 (1949).
5) Stutzer, A., Die Wirkung von Nitrit auf Pflanzen. J. Landw. **54**, 125–138 (1906). Beobachtungen über die Wirkung von Nitrit und von Impferde auf Sojabohnen. J. Landw. **55**, 78–80 (1907).
6) Mevius, W. und Dikussar, S., Nitrite als Stickstoffquelle für höhere Pflanzen. Jahrb. wiss. Botan. **73**, 633-703 (1930).
7) Turtschin, T. W., Zersetzung der Nitrite in mit Basen ungesättigten Böden und das Problem der Nitrifikation. Z. Pflanzenernähr. Düngung Bodenk. **43**, 170–186 (1936).
8) Puri, A. N., Rai, B., and Kapur, R. K., Oxidation of nitrites and oxalates in soils. Soil Sci. **62**, 121–136 (1946).
9) Perciabosco, F., Rosso, V., Staz. Sperim. Agrar. ital. **42**, 5 (1908).
10) Schloesing, M. Th., Nitrates et Nitrites pour engrais. C. Rend. Acad Sci. Paris **141**, 745–746 (1905).

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THE RELEASE OF CARBON DIOXIDE FROM SOILS PERCOLATED WITH VARIOUS ORGANIC MATERIALS

by H. LEES *) and J. W. PORTEOUS

Imperial College of Tropical Agriculture, St. Augustine, Trinidad, B.W.I.

Introduction. Microorganisms supplied with a utilizable source of organic carbon do not, even under aerobic conditions, necessarily convert to CO_2 all the carbon supplied. Some fraction of the carbon is almost invariably elaborated into material that is not immediately katabolized, i.e. some fraction is assimilated. Microbial assimilation, which was first studied by Cook and Stephenson [6], Giesberger [9], Barker [1], and Clifton [2], has now achieved a considerable literature that has recently been well reviewed [10] [3] [19]. However, all results so far published on assimilation seem to have been obtained from experiments with pure cultures of microorganisms; there was therefore some interest in studying assimilation in a naturally-occurring mixed microflora such as the microflora of the soil. The present work, which is an attempt to make such a study, is possibly of more than academic interest since the organic matter of the soil, the 'humus', presumably originates in material initially assimilated by the microflora of the soil.

Materials and methods. In theory, microbial assimilation in soil could be estimated directly from measurements of the amount of extra carbon retained by the soil after the katabolism of an organic compound added to the soil had proceeded to its limit. In practice, the accurate measurement of carbon retention in a medium naturally so highly organic as soil is difficult if not impossible; and if assimilation is to be measured in soil, it must be measured indirectly.

*) Now at the Department of Biological Chemistry, Aberdeen University, Scotland, U.K.

The indirect method used in the present work was the titrimetric estimation of CO_2 released from a known weight of an organic compound percolated through soil [15]; any carbon not released as CO_2 from the organic compound under these conditions was assumed to have been assimilated by the soil microflora. The method can be criticized on the grounds that it takes no account of carbon not assimilated yet released as gases other than CO_2, such as CO or CH_4. On the other hand it seems unlikely that a microflora operating under conditions so highly aerobic as those that obtain in the soil percolators would produce significant amounts of any carbonaceous gas except the fully oxidized CO_2. Any interpretation of the present results in terms of assimilation must, in fact, be based on the *assumption* that the only carbonaceous gas formed from organic compounds katabolized in the soil percolators is CO_2. The same assumption underlies some of the work on assimilation in pure cultures dealt with in the reviews already referred to [3] [10] [19] since a common method of assessing such assimilation has been based on Warburg measurements of the O_2 uptakes of microbial suspensions katabolizing various organic compounds. These measurements were correct only if all the gas produced by the suspensions was absorbed either by the suspension medium or by the alkali, in the centre cup of the Warburg vessel, in other words they were correct only if all the gas produced by the suspensions was CO_2.

Two soils have been studied (*a*) Montserrat clay-silt, a fertile loam from the San Juan Estate in Trinidad (*b*) Ecclesville clay, a poor heavy clay from the Government Settlement at Rio Claro, Trinidad.

Each soil had a pH of 7.0–7.4 and contained 11–14% soil organic matter [12]. The crumb layer, i.e. the rich upper layer of each soil, was sampled, preserved, and prepared for percolation, in the standard way [15]. No sample was preserved for more than 3 weeks. Where experiments were replicated, the replications were always performed on a soil sample different from the one used in the original experiment.

Experiments were begun as follows:

The soil sample was first of all percolated for 16 hours with either water or a solution of any inorganic compound required for the experiment, e.g. KNO_3, NaN_3, the volume of the percolate being 5 ml less than the desired final volume. During this percolation the soil percolator was connected to its collector percolator which contained 100 ml of (approximately) 6×10^{-2} N baryta solution. After 16 hours percolation a 1 ml sample of the baryta was titrated with 7.14×10^{-3} N HCl, phenolphthalein being used as indicator, after which 5 ml of either water or a solution of the required organic compound was added to the soil percolate and percolation proper was begun. Similar HCl titrations were thereafter performed daily on 1 ml samples of the baryta, and the release of CO_2 from the soil and percolate calculated from them [15].

The amount of CO_2 produced from a percolated organic compound *Z* has been calculated as:
'Output of CO_2 from soil percolated with a solution of *Z*' *minus*
'Output of CO_2 from soil percolated with water alone'
the soil weight and percolate volume being the same in each case. The CO_2 so evaluated is hereafter described as "*exogenous* CO_2".

This calculation would give an incorrect value for the amount of CO_2 produced from Z if the mere percolation of Z stimulated or depressed the activities of a soil microflora that produced CO_2 from compounds, other than Z, initially present in the soil. There is no obvious way of overcoming this difficulty. Clifton[3]) has pointed out that "the application of an accurate correction for endogenous respiration remains as an unsolved problem".

From time to time the amount of carbon retained as carbonate and bicarbonate in soil or percolate at the end of an experiment was estimated.

For this purpose, 5 ml concentrated H_2SO_4 was added to the soil-percolate and percolation continued for a further 24 hours. The amount of CO_2 released from soil and percolate during this period, calculated from the fall in HCl titration of the baryta in the collector percolator, was taken to be equivalent to the amount of carbonate and bicarbonate in the soil and percolate. This amount was always found to be less than the equivalent of 1 mg carbon at the end of an experiment; therefore the retention of carbon in an inorganic form by soil or percolate was presumably negligible.

Immobilization of nitrate-nitrogen was followed by methods already described [14]).

Experiments were performed at the ambient temperature of the laboratory, 22–25°C.

Results. The amounts of CO_2 released from soils percolated with either water or glucose solutions are shown in Table I. The amount of exogenously produced CO_2 reached, in 7 days, a steady maximum corresponding to about $\frac{2}{3}$ the amount of CO_2 that would have been obtained had all the glucose been oxidized to CO_2. This value, which was not affected in any regular way by changes in soil weight, percolate volume, or amount of glucose percolated (Table II), is in exact agreement with that given by Cook and Stephenson[6]) who, working with *E. coli*, found that the oxygen uptake of their preparations fell off when about $\frac{2}{3}$ of the oxygen theoretically necessary for

TABLE I

The release of CO_2 from 10 g lots of soils percolated with 100 ml of either water or 0.1% glucose solution. Figures from Montserrat clay-silt (*a*); figures from Ecclesville clay (*b*). Each figure is the mean value from the number of experiments indicated. Standard error of a single observation = 8% of the total carbon percolated as glucose. Total glucose carbon = 40 mg.

	Glucose in percolate	Carbon (mg) evolved as CO_2 after (days)									
		1	2	3	4	5	6	7	8	9	10
	(mg)										
(*a*)	100	12.0	19.9	23.5	26.7	30.4	32.7	34.8	37.7	40.0	41.0
	0	2.4	4.6	6.1	8.0	10.1	10.8	11.5	15.0	15.4	16.3
	Difference	9.6	15.3	17.4	18.7	20.3	21.9	23.3	22.7	24.6	24.7
	No. of experiments	12	12	11	9	11	11	11	7	8	7
(*b*)	100	14.1	23.9	28.3	33.2	37.2	39.6	41.8	46.3	46.8	48.7
	0	5.0	7.6	9.7	11.6	14.4	16.5	17.4	20.7	23.0	23.1
	Difference	9.1	16.3	18.6	21.6	22.8	23.1	24.4	25.6	23.8	25.6
	No. of experiments	12	12	11	9	11	11	11	9	6	7

TABLE II

The effects of changes of soil weight, percolate volume, and amount of glucose percolated, on the exogenous CO_2 outputs of soils percolated with glucose solutions

Weight of soil	Volume of percolate	Glucose percolated	Observed maximum value of exogenous CO_2 output (% of theoretical maximum)	
(g)	(ml)	(mg)	Montserrat clay-silt	Ecclesville clay
5	100	40	60	73
10	100	40	72	66
15	100	40	70	65
10	50	40	61	45
10	100	40	48	55
10	150	40	50	58
10	100	20	65	60
10	100	40	55	50
10	100	60	73	60

complete glucose oxidation had been consumed. The value is also in fair agreement with the observation [16]) that glucose-treated Montserrat clay-silt consumes little over $\frac{1}{2}$ the oxygen required for com-

plete oxidation of the added glucose. Figure 1 shows that the release of exogenous CO_2 followed the equation:

$$\mathrm{Log}_{10}\left(\frac{C}{C-c}\right) - K.t.$$

Where $C = \frac{2}{3}$ of the carbon (mg) percolated as glucose.
$c =$ amount of carbon (mg) released as CO_2 after time 't'.
$K =$ a constant.

The mean value of K, calculated from the slopes of the 'least square' lines of Figure 1, was 1.2×10^{-6} seconds^{-1}.

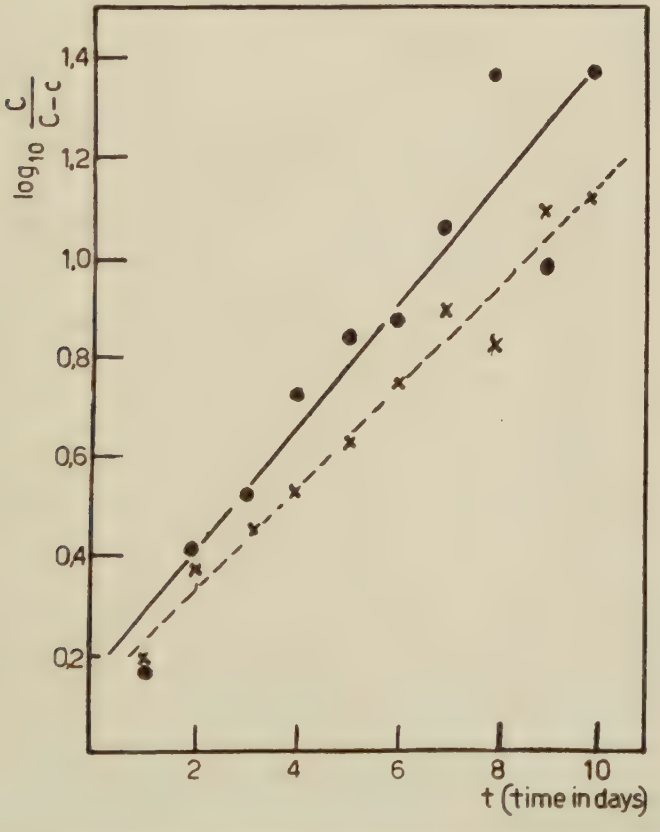

Fig. 1. Exogenous-CO_2 release from 100 mg glucose percolated in 100 ml solution through 10 g lots of soil. C = $\frac{2}{3}$ carbon percolated as glucose; c = carbon released as CO_2 after time 't'. Crosses indicate figures from Montserrat clay-silt; dots indicate figures from Ecclesville clay.

Figures 2 and 3 show the exogenous-CO_2 release curves for five different compounds percolated through both soils. The pattern of

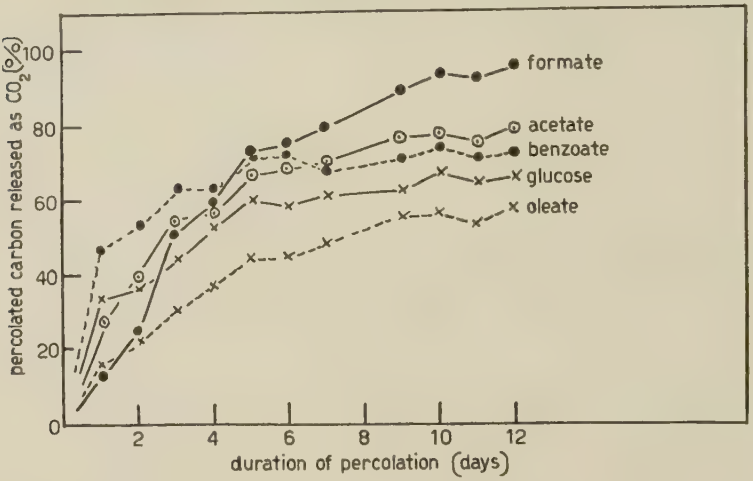

Fig. 2. Exogenous-CO_2 release from various organic compounds percolated in 100 ml solution through 10 g lots Montserrat clay-silt. Total carbon content of each percolate = 40 mg. Each point is the mean of two experiments.

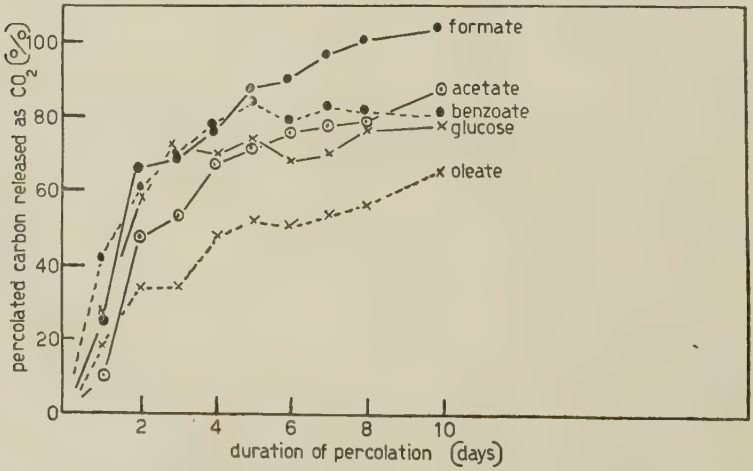

Fig. 3. Exogenous-CO_2 release from various organic compounds percolated in 100 ml solution through 10 g lots of Ecclesville clay. Total carbon content of each percolate = 40 mg. Each point is the mean of two experiments.

the release for any one compound appeared to be characteristic of the compound and was not appreciably influenced by the soil used.

In both soils formate was completely oxidized to CO_2, acetate was $\frac{3}{4}$ oxidized to CO_2, while glucose and benzoate were both $\frac{2}{3}$ oxidized to CO_2. Oleate was very slowly oxidized (possibly because its precipitation as Ca oleate in the soil rendered it less liable to microbial attack) and no characteristic maximum exogenous-CO_2 release can be given for it.

Millimolar concentrations of NaN_3 tended to increase the maximum value of exogenous-CO_2 release from glucose (Table III). This effect, which has been noted on other tropical soils during our investigations, parallells the stimulation of the CO_2 uptake of respiring microbial cells caused by similar concentrations of NaN_3. This effect was noted by Clifton [2], and has since been confirmed by many workers.

TABLE III

The effect of sodium azide on the exogenous CO_2 outputs of 10 g lots of soils percolated with 100 ml 0.1% glucose. Each figure is the mean of two experimental values.

Azide concentration of percolate (M)	Observed maximum value of exogenous CO_2 output (% of theoretical maximum)	
	Montserrat clay-silt	Ecclesville clay
0 (glucose alone)	63	59
2×10^{-3}	74	78
1×10^{-3}	69	72
4×10^{-4}	66	70
2×10^{-4}	62	63

Gray and Taylor [11], working with Canadian soils, showed that CO_2 production from glucose treated soils was increased if nitrate was added to the soils; this result has been confirmed in the present work (Table IV). The exogenous-CO_2 release from glucose in the presence of nitrate was increased by NaN_3 only when the NaN_3 was present in concentrations low enough to allow appreciable immobilization of nitrate-nitrogen (Table V). At higher NaN_3 concentrations, the NaN_3 appeared to replace the nitrate as a stimulator of glucose katabolism. The results of Table V thus suggest that nitrate increases the exogenous-CO_2 release from glucose because glucose acts as a hydrogen donator in the reduction of the nitrate, an essential first step in nitrate immobilization. If the equation:

$$C_6H_{12}O_6 + 3HNO_3 = 6CO_2 + 3NH_3 + 6H_2O$$

is assumed, 2.4 mg carbon (6% of the carbon in 100 mg glucose) should be released for every 1.4 mg nitrate-nitrogen immobilized. The actual immobilizations observed for 2.5 mg nitrate-nitrogen and

TABLE IV

The effect of nitrate, percolated as KNO_3, on the exogenous CO_2 outputs of 10 g lots of soils percolated with 100 ml 0.1% glucose

Nitrate-N in percolate (mg)	Observed maximum value of exogenous CO_2 output (% of theoretical maximum)	
	Montserrat clay-silt	Ecclesville clay
0 (glucose alone)	58	60
1.25	71	64
2.5	67	68
5.0	62	79
10.0	67	76

TABLE V

The effect of sodium azide on nitrate immobilization *) in, and exogenous CO_2 outputs of, 10 g lots of soils percolated with 100 ml 0.1% glucose containing 2.5 mg nitrate-N/100 ml as KNO_3

Azide concentration of percolate (M)	Observed maximum immobilization of nitrate-N (mg)		Observed maximum value of exogenous CO_2 output (% of theoretical maximum)	
	Montserrat clay-silt	Ecclesville clay	Montserrat clay-silt	Ecclesville clay
0	2.3	1.8	62	57
1×10^{-3}	0.3	0.2	64	55
5×10^{-4}	1.3	0.8	72	61
2×10^{-4}	1.6	0.9	69	58
8×10^{-5}	2.2	0.9	70	55

*) Studied by methods described in reference 14.

100 mg glucose were (Table V) 2.3 mg nitrate-nitrogen for Montserrat clay-silt and 1.8 mg nitrate-nitrogen for Ecclesville clay, corresponding to 10% and 8% extra exogenous-CO_2 release respectively. The observed figures for extra exogenous-CO_2 release under these conditions were (Table IV) 9% and 8%.

Discussion. Glucose, Na acetate, Na benzoate, and Na oleate, were not completely oxidized to CO_2 when they were percolated as water solutions through two tropical soils. If it be assumed that the

percolated organic carbon not released as CO_2 was assimilated by the soil microflora, then the fractional assimilations of carbon were, $\frac{1}{3}$ for glucose, $\frac{1}{4}$ for acetate, $\frac{1}{8}$ for benzoate, and possibly about $\frac{1}{3}$ for oleate. Since microorganisms differ somewhat in the extent to which they assimilate the carbon of any given organic compound [20], [9], [3], no precise comparison of the present results with published results on assimilation in pure culture is possible. Nevertheless it may be said that the figures suggested for assimilation by the soil microflora are of the same order as those observed for assimilation in pure culture [3] [10] [19]. Formate is apparently not an assimilable radicle [3] [6]; complete oxidation of formate to CO_2 by the soil microflora was observed in the present work. The formule, given in the Materials and Methods section, by means of which exogenous-CO_2 outputs have been calculated is therefore apparently a reliable one.

It has been suggested [5] that if the general assimilatory process is represented as:

Substrate $+O_2$ (yielding energy) $\rightarrow$ assimilated material $+CO_2+H_2O$

then the efficiency of synthesis of assimilated material depends upon the relative free energies of the two halves of the reaction (*a*) production of assimilated material (*b*) production of CO_2. The material assimilated from the compounds investigated in the present work was probably largely carbohydrate [7] [8]; if therefore we assume that the katabolism of a given material followed the same steps in both soils, and that the assimilated material was built up from corresponding intermediates in both soils, the release of CO_2 from a given compound should have been characteristic of the compound rather than of the soil katabolizing it. This has been observed (Figures 2 and 3).

Sodium azide increased the amount of CO_2 produced from percolated glucose, an expected result if azide inhibited assimilative processes in soil as it does in culture [4] [2] [5] [23]. The inhibition is probably due to an uncoupling of hydrogen-transfer reactions from the generation of phosphate-bond energy [13] [21] [18]. Azide did, in fact, inhibit nitrogen assimilation at low concentrations in the soils tested (Table V); in the same two soils azide was equally inhibitory towards nitrification [17], a process in which carbon assimilation is obligatory [22].

Only two soils have been studied, the results must therefore be looked on as preliminary. Nevertheless it should seem that the modified percolation technique may be of value in the study of some

aspects of carbon metablism in soils. This technique is to be used in work now projected on the formation and breakdown of soil organic matter in temperate soils.

Summary

1) The output of carbon dioxide from soils percolated with different organic materials has been studied by the percolation technique.

2) Assimilation appears to take place from glucose, acetate, benzoate, oleate, but not from formate.

3) Sodium azide appears to inhibit assimilative activity in soil.

4) The significance of the results is discussed.

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REFERENCES

1) Barker, H. A., Oxidative metabolism of the colorless alga *Prototheca zopfi*. J. Cellular Comp. Physiol. **8**, 231 (1936).
2) Clifton, C. E., On the possibility of preventing assimilation in respiring cells. Enzymologia **4**, 246–253 (1937).
3) Clifton, C. E., Microbial assimilations. Adv. Enzymol. **6**, 269–308 (1946).
4) Clifton, C. E. and Logan, W. A., Oxidative assimilation of lactic acid by *Escherichia coli*. Proc. Soc. Exp. Biol. N.Y. **38**, 619–622 (1938).
5) Clifton, C. E. and Logan, W. A., On the relation between assimilation and respiration in suspensions and in cultures of *Escherichie coli*. J. Bact. **37**, 523–540 (1939).
6) Cook, R. P. and Stephenson, M., Bacterial oxidations. Biochem. J. **22**, 1368–1386 (1928).
7) Forsyth, W. G. C., Studies on the more soluble complexes of soil organic matter. Biochem. J. **41**, 176–181 (1947).
8) Forsyth, W. G. C., Carbohydrate metabolism in soil. Chem. and Ind., 515–519 (1948).
9) Giesberger, G., Beitrage zur Kenntnis der Gattung Spirillum Ehgb. Dissertation, Utrecht 1936 (quoted by 2, 6 und 19).
10) Goddard, D. R., Physical Chemistry of Cells and Tissues. (Edited R. Höber). Churchill, London, 1945.
11) Grey, P. H. H. and Taylor, C. B., Aerobic decomposition of glucose in podsol soils. Can. J. Research (*c*) **17**, 109–124 (1939).
12) Hardy, F., A note on the determination of soil organic matter. J. Agric. Sci. **19**, 727–733 (1929).
13) Lardy, H. A. and Elvehjem, C. A., Biological oxidations and reductions. Ann. Rev. Biochem. **14**, 1–28 (1945).
14) Lees, H., Immobilization of mineral nitrogen by different organic materials. Biochem. J., 531–534 (1948).
15) Lees, H., The soil percolation technique. Plant and Soil **1**, 221–239, 1949.
16) Lees, H., A simple apparatus for measuring the oxygen uptake of soils. Plant and Soil **2**, 123–128 (1949).

17) Lees, H. and Porteous, J. W., in preparation.
18) Loomis, W. F. and Lipmann, F., Inhibition of phosphorylation by azids in kidney homogenate. J. biol. Chem. **179**, 503–504 (1949).
19) McElroy, W. D., The mechanisms of inhibition of cellular activity by narcotics. Quart. Rev. Biol. **22**, 25–58 (1947).
20) Pickett, M. J. and Clifton, C. E., Effect of selective poisons on utilization of glucose by yeast. Proc. Soc. Exp. Biol. Med. **46**, 443–445 (1941).
21) Spiegelman, S., Kamen, M. D. and Sussman, M., Phosphate metabolism and the dissociation of anaerobic glycolysis from synthetis in the presence of sodium azide. Arch. Biochem. **18**, 409–436 (1948).
22) Stephenson, M., Bacterial Metabolism, Longmans, London, 1949.
23) Winzler, R. J., A comparative study of the effects of cyanide, azide and carbon monoxide on the respiration of bakers yeast. J. Cellular Comp. Physiol. **21**, 229–252 (1943).

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CATION EXCHANGE PROPERTIES OF PLANT ROOT SURFACES

by D. E. WILLIAMS and N. T. COLEMAN *)

Division of Soils, University of California

Introduction. In evaluating certain experimental data concerning interactions between plant roots and soils or clay suspensions it has been convenient to invoke the concept of an ion double layer at root surfaces. As has been pointed out by Jenny [4]), many experiments relating to cation accumulation by, or depletion from, plant roots can be most satisfactorily explained on the basis of an ion exchange between the cation exchange material in the substrate and the root itself. Such a mechanism presupposes surfaces on plant roots capable of cation exchange. The fact that such contact phenomena have been demonstrated is in itself excellent evidence for the existence of an ionic double layer on the root. The present investigation was launched in an effort to secure further evidence for the existence of cation exchange on surfaces of plant roots and to define in an experimental way the properties of these surfaces.

The suspension effect. The "suspension effect" has long been regarded as a manifestation of a H-ion swarm associated with solid-liquid interfaces in a colloidal system [8]). It is probably more nearly correct to state that the "suspension effect" is a reflection of an ionic double layer at the boundary between a charged particle and a liquid, and is not due exclusively to H-ions. In any event, the solid

*) Assistant Soil Chemist, A. E. C. Research Fellow, respectively. Assistance of T. R. Nielsen with many of the analyses is acknowledged.

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phase of an electro-negative system such as a large H-resin grain will have a pH *) lower than the pH of its supernatent liquid.

TABLE I

pH values given by plant roots immersed in CO_2-saturated H_2O				
Plant	pH of supernatent liquid	pH at root surface		
		Maximum	Mean †)	Minimum
Atlas barley	4.00	3.60	3.30	3.00
Sacramento barley	4.05	3.40	3.17	2.80
Tomato	4.10	3.70	3.02	2.10
Corn	3.95	3.75	3.40	2.90
Cotton	4.02	3.20	2.91	2.20
Lettuce	4.10	3.20	2.91	2.60
Soybean	4.00	3.60	3.16	2.85
Peanut	4.00	3.50	3.15	2.50
Sugar beet	4.00	3.60	3.27	3.00
Celery	3.95	3.20	2.92	2.75
Tobacco	4.10	3.60	2.92	2.00

†) Based on 15–20 individual measurements.

That this is also the case for the roots of several species of plants is shown in table I. Plants were grown in $\frac{1}{4}$ Hoagland nutrient solution and were then transferred to tap water for several weeks. Immediately prior to a given experiment, plants were removed from the tap water and the roots were thoroughly washed in distilled water. The roots were then immersed successively in three batches of CO_2-saturated water, remaining in each change of water for 15 minutes. Finally, a sufficient number of plants to yield 2–3 g of roots was transferred to a small beaker containing CO_2-saturated H_2O, and pH determinations were made with a glass electrode -saturated calomel electrode assembly. The pH of the supernatent liquid was measured in the usual manner. Then, by manipulation of the container, the roots were gently pressed against the electrodes so as to measure the pH of the root surfaces. Some fifteen to twenty such measurements were made on a given set of roots.

Table I lists the maximum, minimum, and mean pH values given by the roots of eleven plant species. The maxima and minima give

*) It is realized that pH measurement in colloidal systems is not strictly analogous to pH measurement in true solutions, but the widespread use of the term "pH" to describe the potential observed when a glass electrode and a salt bridge are placed in a collodial system makes it desirable to retain this terminology.

the range of values obtained, while the mean indicates the tendency toward either high or low values.

Roots of all the plant species studied yielded pH values considerably below the pH values of the supernatent liquid, which was, in all cases, CO_2-saturated H_2O. According to the general interpretation of the suspension effect, this would indicate a H-ion swarm associated with outerroot surfaces. It has been pointed out earlier that the suspension effect is merely indicative of a cation double layer at a negatively charged surface, but since the roots had been repeatedly washed in CO_2-saturated water, presumably the population of the double layer in this case was largely H^+.

In any series of pH determinations on a given set of plant roots, a considerable range of values was obtained. For the case of tobacco roots the variations in pH are as follows: 3.6, 2.6, 3.3, 3.0, 2.9, 3.3, 2.0, 3.1, 2.7, 2.6, 3.1, 3.0, 2.7, 2.7, 3.1. The variability might well be due to variations in charge density and degree of dissociation on the root surface.

pH values given by the roots of various plants showed some species differentiation. Referring to table I, barley, soybean, and corn roots gave consistently higher pH values than cotton and tobacco roots. Since considerable uncertainty exists with respect to assigning a given value of pH as characteristic of a given root, such species differences as were observed cannot be quantitatively assessed at the present time.

Table II compares pH values given by roots at room temperature and at 0°C, and by living roots and roots which had been killed by one hour immersion in ether. Considering the first portion, of table II,

TABLE II

pH of plant roots as affected by temperature and ether treatment

Plant	Treatment	pH of supernatent liquid	pH at root surface		
			Maximum	Mean	Minimum
Atlas.	25°C	4.10	3.70	3.46	3.25
barley	0°C	4.25	3.80	3.50	3.20
Sacramento	25°C	4.05	3.50	3.21	3.00
barley	0°C	4.10	3.50	3.36	3.25
Tomato	25°C	4.05	3.90	3.69	3.50
	0°C	4.20	3.80	3.65	3.50
Celery	living	3.95	3.20	2.92	2.75
	ether-killed	4.00	3.50	2.93	2.70

it is apparent that there is no relation between pH and the temperature of measurement, since identical results were obtained at both temperatures. Since plant metabolic processes are known to occur extremely slowly at 0°C, this is strong evidence against any direct relationship between root metabolism and the constitution of the cation double layer. This contention is supported further by the data in table II, comparing pH values given by living roots and ether killed roots. Since the data obtained are nearly identical, regardless of the condition of the roots, it seems that the negative charge possessed by root surfaces is associated either with non-living portions or with compounds which undergo no immediate change on the diminution or cessation of metabolism.

TABLE III

pH of plant roots as affected by electrolytes

Plant	Ambient solution	pH of solution	pH at root surface		
			Maximum	Mean	Minimum
Celery . . .	H_2O-CO_2	3.95	3.20	2.92	2.75
	HAc	3.70	3.30	2.95	2.70
Tomato . .	H_2O-KCl	4.50	4.50	4.50	4.50
	H_2O	5.90	3.90	3.59	3.40
	H_2O-CO_2	4.00	3.50	3.27	3.10
	HCl	4.15	3.50	3.40	3.10
	HCl	3.15	3.30	3.06	2.90
Cotton . . .	H_2O-KCl	4.75	4.75	4.75	4.75
	H_2O	5.90	3.80	3.59	3.40
	H_2O-CO_2	4.00	3.60	3.33	3.00
	HCl	4.20	3.50	3.45	3.25
	HCl	3.15	2.90	2.79	2.70
Tobacco . .	H_2O-KCl	4.80	4.80	4.80	4.80
	H_2O	5.90	3.85	3.71	3.50
	H_2O-CO_2	4.05	3.80	3.26	2.90
	HCl	4.15	3.60	3.28	3.00
	HCl	3.20	3.10	2.91	2.70

That pH values obtained with plant roots are not restricted to CO_2 in the bathing solution is shown in table III. Here are listed pH values given by roots in CO_2-saturated water, in double-distilled water, in HCl solutions, and in acetic acid solution. In all cases the roots were washed in three changes of the solution in question and then immersed in a fresh supply of that solution for pH determinations. A suspension effect was manifested in all cases regardless of

the solution in which the roots were immersed. This "suspension effect" shown by plant roots in various salt-free solutions is analogous to that observed in the case of clays and other cation exchange materials, and shows the presence of a cation double layer at the solid-solution interface.

Exchange acidity. H-saturated plant roots also show the phenomenon of exchange acidity when treated with neutral salts. To demonstrate exchange acidity, roots were H-saturated by treatment with CO_2-saturated water as described earlier, washed with double-distilled water to remove excess CO_2, and dipped into salt solutions for 10 sec. The pH of the salt solution was measured before and after contact with the roots. In all cases the pH of the salt solution was lowered by contact with the H-saturated roots. In similar experiments, H-saturated roots were washed and transferred to double distilled water, and the pH of both ambient liquid and of root surfaces was determined before and after the addition of KCl.

Table III presents data which show replacement of root H-ions by cations of a neutral salt. The sections of table III labeled "H_2O" and "H_2O–KCl" should be compared. In the case of tomato roots, for example, H-roots in H_2O gave a mean pH of 3.59, while the supernatent liquid had a pH of 5.9. After the addition of 15 ml of 0.5 N KCl solution of pH 7 to the water-root system the situation depicted under "H_2O -KCl" prevailed. Here the pH of the water had dropped to 4.5, and the pH of the roots had risen to 4.5. Exchange acidity developed immediately when the KCl solution was added to the liquid bathing the roots, indicating an outer surface reaction. The depression in the pH of the supernatent liquid may be attributed to the replacement of root H-ions by cations of the neutral salt. The disappearance of the suspension effect in the presence of the excess electrolyte in part is due to the formation of a K-root surface.

In order to show conclusively that the effect of a neutral salt on H-saturated plant roots consists in release of H-ions from the roots, potentiometric titrations with NaOH were performed with 4 systems, Malic acid, CO_2-saturated water, H_2SO_4 in K_2SO_4 solution, and K_2SO_4 solution into which H-saturated roots has been dipped for 10 seconds. The latter curve depicted by the small circles in figure 1 was identical with that for H_2SO_4 in K_2SO_4, and in no way resembled that for CO_2 water or that given by an organic acid which might be

excreted from roots. The evidence is clear that there can be an actual instantaneous replacement of H-ions from root surfaces by other cations.

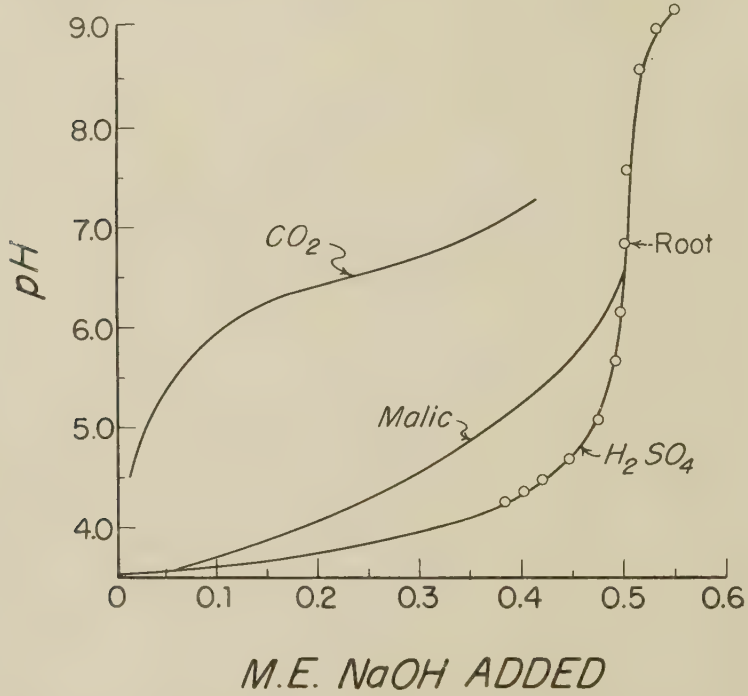

Fig. 1. Titration of H^+ replaced from plant root surfaces by K_2SO_4. Titration curves of other acids are shown for comparison.

Cation exchange capacity of roots. The "suspension effect" of plant roots and their interaction with neutral salts with the development of exchange acidity are indicative of the existence of ion swarms on root surfaces. Capacities of the roots for binding cations under a given set of conditions were determined by saturating roots with Ba^{++}, or NH_4^+, or H^+ by means of a 10 sec. immersion in neutral salt or acid solution, followed by a 10 sec. immersion in a replacing solution.

Saturation with Ba^{++} was effected in a 0.03 N Barium acetate solution of pH 7. After thorough washing in distilled water, Ba^{++} was replaced from the roots with 0.03 N $CaCl_2$ at pH 7, and was determined as described by M e h l i c h [6]). Roots were NH_4-saturated

in 0.03 N Ammonium acetate at pH 7, washed and dipped into 0.01 N acetic acid replacing solution. NH_4^+ was determined in the replacing solution by Nesslerization. H-saturation of roots was accomplished by treatment with CO_2-saturated water, as described earlier. H-saturated washed roots were dipped for 10 secs. into 0.5 N $BaCl_2$ solution at pH 8.2, removed, and the solution was titrated with NaOH to the original pH.

Preliminary experiments involving varying times of contact between roots and saturating and replacing solutions showed exchange capacities for a given set of roots to be relatively constant as long as contact with the two solutions was on the order of 30 secs. or less. With longer times of contact, the amount of ion taken up and released became larger. With Sacramento barley roots, for example, exchange capacities measured by 5, 10, 20, and 30 sec. immersions in Ba^{++} and 30 sec. replacement with Ca^{++} were respectively, 0.562, 0.562, 0.567, and 0.571 m.e. per 100 grams of fresh root. For the same plants, Ba^{-} released by 1 hour replacement following 1 hour saturation amounted to 1.541 m.e. per 100 grams. The fact that relatively large amounts of Ba are adsorbed and replaced in very short periods of time indicates that the reactions take place at, or near, the outer surface of the roots. The larger amounts of ion uptake and release during 1 hour periods probably reflects movement of ions into and out of the interior of the roots.

Table IV lists cation exchange capacities measured for the roots of various plant species by means of successive saturation and desatu-

TABLE IV

Cation exchange capacity of plant roots			
Cation exchange capacity of roots; m.e./100 g fresh weight			
Plant	Ba^{++}	NH_4^+	H^+
Barley (Atlas)	0.545	0.473	0.820
Barley (Sacramento)	0.448	0.480	0.731
Cotton	0.396	0.469	0.395
Corn	0.312	0.191	0.245 *)
Soybean	0.633	0.330	0.550 *)
Tobacco	0.257	0.287	0.532
Lettuce	0.401	0.313	0.726
Tomato	0.263	0.277	0.420
Sugar beet	1.308	—	—
Peanut	0.470	0.529	0.592

*) Determined by titration to BTB end point at pH 6.5.

ration for 10 second periods. These data are of interest largely because they demonstrate a measurable and reproducible cation exchange capacity for root surfaces and because measurement with different ions gives results of the same order of magnitude. At the present time, no physiologic and morphologic significance can be attached to the differences in exchange capacity exhibited by the various plant species. The measured cation exchange capacities, while small when expressed on a gross weight basis, must be very large when only the surface portion of the roots is considered *).

The exchange capacities for Ba^{++} and NH_4^+ at pH 7 are quite similar in most cases, though they do not agree nearly so closely as do duplicate determinations involving one of these ions. For corn, soybean, and possibly lettuce, Ba^{++} adsorption is significantly greater than NH_4^+ adsorption.

The values for exchangeable H^+, determined by titration of H^+ replaced at pH 8.2, are, in general, higher than the amounts of exchangeable Ba^{++} and NH_4^+ at pH 7. This is explicable in the light of similar results with other cation exchange materials whose cation exchange capacities increase as the pH becomes higher.

That the retention of cations at outer root surfaces is a reversible exchange process was shown by the following experiment. Ten cm segments of excised barley roots were immersed for 10 seconds in 0.01 N CsCl solution tagged with Cs^{137}. The root segments were removed, washed with distilled water, and divided into 3 portions. One portion, No. 1, underwent no further treatment. The second portion, No. 2, was dipped for 10 seconds into a 0.01 N solution of CsCl without Cs*, and the third was dipped for 10 seconds into 0.03 N $CaCl_2$. The activities, in counts per second, of 20 root segments from each group were as follows: 1. 73.84; 2. 5.05; 3. 2.22. Ninety-three per cent of the Cs* adsorbed by the barley roots underwent rapid isotopic exchange with CsCl solution, while 97% of the root surface Cs^+ was exchanged in $CaCl_2$ solution. In a similar experiment 98% of the adsorbed methylene blue (Me. B.) was exchanged from barley roots in $BaCl_2$ solution.

Cations which become associated with outer root surfaces during the short periods of contact studied here are freely exchangeable.

*) In a recent paper Mattson and co-workers (Annals Agr. College Sweden, **16**, 457–484) also conclude that root surfaces and especially root tips have "very high" cation adsorption capacities.

Ion binding by protoplasm, with the formation of chelated structures [3]), does not occur in the rapid initial exchange reaction between inorganic cations and plant roots.

The rapidity of the exchange reactions between plant roots and salt solutions indicates that a surface process is under investigation. Presumably such a reaction, while it would involve some product of plant metabolism, would not be directly dependent on any simultaneously occurring physiological process. This contention is borne out by the evidence presented in table V. The top portion of table V

TABLE V

Cation exchange capacities of living and ether-killed plant roots

Plant	Condition	M.e. Ba^{++} Exchanged per 100 grams
Atlas barley . . .	living, 25°C	0.562
	living, 0°C	0.459
Tomato.	living	0.263
	killed	0.318
Tobacco.	living	0.257
	killed	0.259
Peanut	living	0.470
	killed	0.484

compares the cation exchange capacity of Atlas barley roots as measured at 0°C and at 25°C. The values are similar. The rest of table V concerns exchange capacities of three species of plant roots as measured when the roots are attached to living plants and measured after the roots had been excised and killed in ether. The nearly identical values given by living roots and dead roots of a given species strongly indicates that the cation exchange properties measured are associated with non-living portions of the roots, presumably the exterior walls of the epidermal cells. It is realized that the over-all mechanism of ion accumulation by plant roots is intimately related to metabolic activity [2]). This fact, however, in no way makes it unreasonable to suppose that an ion exchange process could play a role in the accumulation process.

The lyotropic series in ion exchange at root surfaces. Information concerning the relative replacing abilities of the alkali and alkaline

earth chlorides at the ion double layer of tobacco roots is given in fig. 2. Roots of several plant species were saturated with methylene blue (Me. B.) by dipping them into a 0.1% solution of the chloride salt of this large cation. The deep blue roots were then washed ten times with distilled water and were dipped for 10 seconds into the appropriate salt solution. The same roots were again saturated with Me. B. and washed, and were then dipped into another salt solution. This process of saturation and replacement was continued until Me. B. replacement by all concentrations of all salts had been made. Me. B. in the various solutions was determined by measuring their transmittancy, using a red filter.

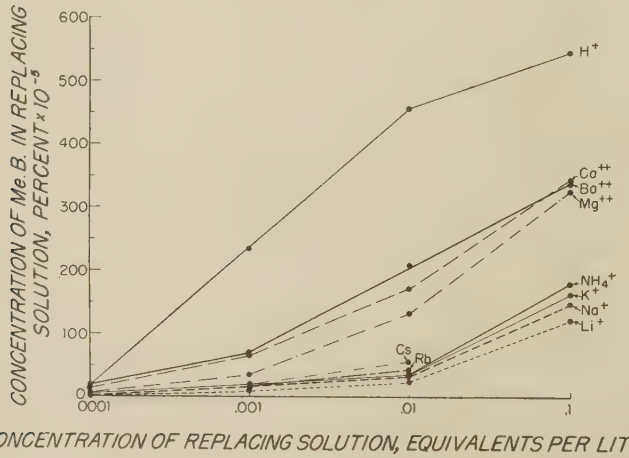

Fig. 2. Replacement of Me. B. from Tobacco roots by chloride salts of various concentrations. The lyotropic series holds at low salt concentrations (10^{-4}) which range is similar to that of soil solution.

For all the salts used the amount of Me. B. appearing in the replacing solution increased with increasing concentration. This is to be expected from mass action effects. At any given concentration, the replacing abilities of the cations decreased in the order:

$$H^+ > Ba^{++} > Ca^{++} > Mg^{++} > Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+$$

This pattern of replacement was consistent for all plants studied. The results for only one species, tobacco, is presented in detail in figure 2.

An experiment was performed in which was measured the replacement of H^+ from tobacco roots by various cations. The order of replacing abilities was identical with that in the Me. B. experiment. This is, in general, the lyotropic series to be expected for ion retention at a cation exchange surface. The relative retention of the ions in the series, other than H^+, are related to charge and ion hydration [1]). The order is not, however, that in which ions are accumulated by plants. Barley roots, for example, accumulate in the cell sap much greater quantities of K than Ca. Evidently, entry of cations into the root double layer proceeds according to the rules of exchange adsorption. The specific mechanism for physiologic differentiation of ion uptake must be placed at a later stage in the process of ion accumulation.

Root surface zones active in ion exchange. The fact that in a series of pH measurements on plant roots both high and low readings were obtained, indicates that root surfaces may consist of both active and relatively inactive portions insofar as cation exchange is concerned. Work of other investigators [2]) [7]) has shown that zones in the vicinity of the root tips are most active in ion accumulation. The experiments summarized in figure 3 give some insight as to which root surface zones have the greatest cation exchange capacities. Roots of corn, bean, and barley were immersed for 10 seconds in 0.01 CsCl solution tagged with Cs^{137}, removed and washed with distilled water, and the activity of various segments was determined with the aid of a Geiger-Müller counter. The curves labeled "corn" and "bean" show the variation in the amount of Cs* taken up by roots of these plants as related to distance from the tip. In both cases, the activity of the first 2 mm segment, containing the root tip, was higher than that of other segments from the same root. This indicates that cation exchange, as well as ion accumulation is greatest immediately adjacent to the growing tip. Microscopic examination of the corn roots used in this experiment showed them to be free of root hairs and lateral buds. The bean roots, however, showed definite initiation of lateral buds in the second cm from the tip, and it is probable that the consistent increase in the amount of Cs* on bean root surfaces from 0.8–2 cm is correlated with this lateral initiation.

Greater accumulation of ions by meristematic regions as compared with the mass of the root is generally ascribed to respiratory

differences. Since it has been shown that cation adsorption on root surfaces is not directly dependent on metabolism, this explanation

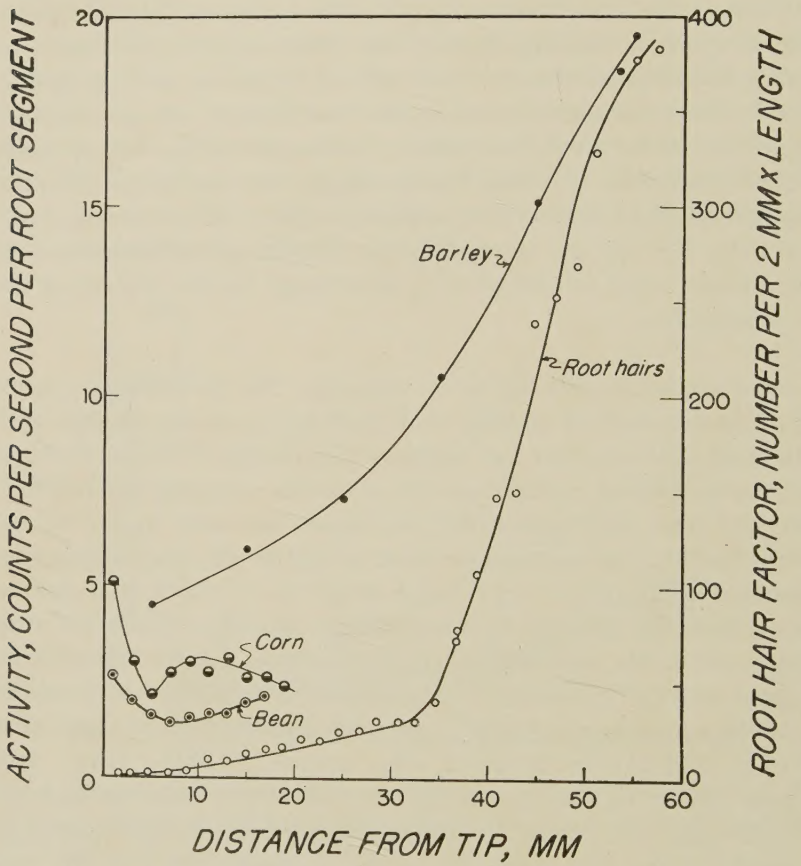

Fig. 3. Lateral distribution of adsorbed Cs* on root surfaces.

cannot apply here. It is probable that the nature of the cell walls in regions of cell division and enlargement is not the same as in mature cells, and that differences in the root epidermis are reflected by differences in surface exchange properties.

The pattern of Cs* adsorption by young barley roots is also shown in figure 3. In this case, activity is least for the first 1 cm portion and increases from this first portion back. Figure 3 also shows the pattern of root hair distribution on the barley roots used in this experiment.

The "root hair factor", one of the ordinates, is the product of the number of root hairs extending from a given 2 mm root segment, multiplied by the average length of the hairs on this segment. Assuming a constant root hair diameter, this root hair factor is proportional to root hair surface. The root hair factor was obtained by microscopic examination of the roots in question.

There is a definite correlation between root hair distribution and the surface retention of Cs^+. Certainly the proliferation of root hairs by a root segment increases considerably the surfaces exposed by that segment to the root environment. The walls of root hairs are said to be largely pectic in nature, and the possibility of finding cation exchange spots on pectic surfaces is great.

Discussion. All of the three classes of experiments performed indicate the existence of an ionic double layer at root surface-environment interfaces. L u n d e g å r d h [5]) showed root tips and regions of root hair proliferation to yield high negative potentials, and inferred a greater charge density in these zones as compared with the rest of the root. While his data agree with the surface distribution of Cs* on roots, it should be pointed out that L u n d e g å r d h assigned the seat of exchange adsorption to the cytoplasm exclusively. This is not in accord with the data presented here.

The full significance of this work with regard to the larger aspects of ion adsorption by plant roots is difficult to assess at the present time. It may be postulated that entry of cations into the ionic double layer associated with root surfaces and cell walls is the first step in ion adsorption. The validity of this postulate must be further examined. Contact uptake and depletion of cations [4]) can be explained most satisfactorily by supposing the intervention of the ionic double layers at root surfaces.

Summary

Plant roots show the "suspension effect" and interact with neutral salts with the development of exchange acidity, thus indicating the existence of a cation double layer associated with root surface. Plant root surfaces possess cation exchange capacities which may be measured by the adsorption and release of various cations. H-ions are associated with root surfaces in exchangeable form, and may be replaced by other cations.

These phenomena do not depend directly upon root metabolism, since the

same results are obtained at 0°C and 25°C, with living roots and with ether-killed roots.

Entry of cations into the root double layer follows the type of lyotropic series observed with many inorganic and organic exchange materials. The lyotropic series, in decreasing order of replacing ability is:

$$H^+ > Ba^{++} > Ca^{++} > Mg^{++} > Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+.$$

Root tips and root hair zones are the most active root portions insofar as surface exchange properties are concerned.

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REFERENCES

1) Gieseking, J. E. and Jenny, Hans, Behavior of polyvalent cations in base exchange. Soil Sci. **42**, 273–280 (1936).
2) Hoagland, D. R. and Broyer, T. C., Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. **25**, 865–880 (1942).
3) Jacobson, Louis and Overstreet, Roy, A study of the mechanism of ion absorption by plant roots using radioactive elements. Amer. Jour. Bot. **34**, 415–420 (1947).
4) Jenny, Hans, Contact phenomena between adsorbents and their significance in the mineral nutrition of plants. Plant Nutrition Symposium, University of Wisconsin Press, 1949.
5) Lundegårdh, Henrik, Investigations as to the absorption and accumulation of inorganic ions. Ann. Agric. Col. Sweden **8**, 234–404 (1940).
6) Mehlich, A., Determination of cation- and anion-exchange properties of soils. Soil Sci. **66**, 429–445 (1948).
7) Stewart, F. E., Prevot, C. P., and Harrison, J.A., Absorption and accumulation of rubidium bromide by barley roots-Localization in the root of cation accumulation and of transfer to the shoot. Plant Physiol. **17**, 411–421 (1942).
8) Wiegner, G., Some physico-chemical properties of clays. Soc. of Chem. Ind. **50** 55–71 (1931).